Nicotine-induced resistance of non-small cell lung cancer to treatment – possible mechanisms*

Indukowana przez nikotynę oporność niedrobnokomórkowego raka płuca na terapię – możliwe mechanizmy

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Summary

Cigarette smoking is the leading risk factor of lung cancer. Data from several clinical studies suggest that continuation of smoking during therapy of tobacco-related cancers is associated with lower response rates to chemotherapy and/or radiotherapy, and even with decreased survival. Although nicotine – an addictive component of tobacco – is not a carcinogen, it may influence cancer development and progression or effectiveness of anti-cancer therapy. Several *in vitro* and *in vivo* trials have evaluated the influence of nicotine on lung cancer cells. The best known mechanisms by which nicotine impacts cancer biology involve suppression of apoptosis induced by certain drugs or radiation, promotion of proliferation, angiogenesis, invasion and migration of cancer cells. This effect is mainly mediated by membranous nicotinic acetylcholine receptors whose stimulation leads to sustained activation of such intracellular pathways as PI3K/Akt/mTOR, RAS/RAF/MEK/ERK and JAK/STAT, induction of NF-κB activity, enhanced transcription of mitogenic promoters, inhibition of the mitochondrial death pathway or stimulation of pro-angiogenic factors. We herein summarize the mechanisms underlying nicotine's influence on biology of lung cancer cells and the effectiveness of anti-cancer therapy.

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Abbreviations: **Akt** – serine/threonine protein kinase Akt (also known as protein kinase B); **A549** – human alveolar adenocarcinoma cell line; **BAD** – BCL-2-associated death promoter; **BAX** – BCL2-associated X *protein*; **Bcl-X$_L$** – Bcl2-like protein 1; **BCL2** – B-cell lymphoma 2 protein; **BEAS-2B** – immortalized human bronchial epithelial cell line; **β-AR** – beta adrenergic receptor; **Chk2** – checkpoint kinase 2; **CRT** – chemoradiotherapy; **CT** – chemotherapy; **DNA** – deoxyribonucleic acid; **EGF** – epidermal

growth factor; **EGFR** – epidermal growth factor receptor; **EMT** – epithelial to mesenchymal transition; **EPC** – endothelial progenitor cells; **ERK1/2** – extracellular signal-regulated protein kinases 1 and 2; **Glut**-1 – glucose transporter 1; **HIF-1α** – hypoxia-inducible factor 1-alpha; **IAPs** – inhibitor of apoptosis proteins; **IGF** – insulin-like growth factor; **IKBKE** – inhibitor of nuclear factor kappa-B kinase subunit epsilon; **JAK** – Janus kinase; **MAPK** – mitogen activated protein kinase; **MAP2K1** – mitogen-activated protein kinase kinase 1; **MCP-1** – monocyte chemotactic protein-1; **MDM2** – mouse double minute 2 homologue; **mTOR** – mammalian target of rapamycin; **MT** – mutated; **nAChR** – nicotinic acetylcholine receptor; **NF-κB** – nuclear factor kappa B; **NIH-3T3** – mouse embryonic fibroblast cell line; **NNK** – 4-(methylnitrosoamino)-1-(3-pyridyl)-1-butanone; **NNN** – N-nitrosonornicotine; **NSCLC** – non-small cell lung cancer; **PI3K** – phosphoinositide 3-kinase; **PKA** – *protein* kinase A; **PKC** – protein *kinase* C; **PKCiota** – protein kinase Ciota; **p107** – retinoblastoma-like protein 1; **p130** – retinoblastoma-like protein 2; **RAR** – retinoic acid receptor; **Rb** – retinoblastoma tumor protein; **ROS** – reactive oxygen species; **RT** – radiotherapy; **siRNA** – small interfering RNA; **Src** – tyrosine-protein kinase Src; **STAT** – signal transducer and activator of transcription; **TKI** – tyrosine-kinase inhibitor; **TNF-α** – tumor necrosis factor α; **VEGF** – vascular endothelial growth factor; **WT** – wild type; **XIAP** – X-linked inhibitor of apoptosis; **Raf** – Raf serine/threonine kinase.

INTRODUCTION

Lung cancer is the leading cause of cancer-related death worldwide. Non-small cell lung cancer (NSCLC) accounts for 80-90% of all lung cancers and has a strong etiologic association with smoking. Poor prognosis is related to high frequency of advanced stage at diagnosis and the inherent therapeutic resistance of cancer cells to treatment.

Cigarette smoking generates over 6000 compounds, 60 of which are known to be carcinogens, e.g. polycyclic aromatic hydrocarbons, nitrosamines (NNN, NNK) and aldehydes, which induce formation of DNA adducts, thereby causing mutations in vital genes and genetic instability. Although nicotine – an addictive component of tobacco – is not a carcinogen, it has been suggested that it may also lead to the sustained activation of molecular pathways responsible for growth promotion and, finally, to the development of new tumors [10,20,24,26]. Moreover, smoking constituents – mainly nicotine – act as immunosuppressors by inhibiting both innate and adaptive immune responses, resulting in higher risk of various cancers [35]. Concerning those mechanisms, nicotine is known as a survival agonist that inhibits apoptosis induced e.g. by chemotherapeutic agents; therefore it has been established that nicotine influences the responsiveness of cancer cells to treatment [40].

 Several retrospective studies have shown that continuing to smoke during anti-cancer therapy of tobacco-related neoplasms, such as lung cancer and head-and-neck cancer, is associated with lower response rates to chemotherapy and/or radiation, and in some cases, even in decreased survival [4,37]. Retrospective analysis of data from the Memorial Sloan-Kettering Cancer Center tumor registry in 1997 also demonstrated that smoking had a negative impact on survival of both tobacco-related and non-tobacco-related cancers [44]. In addition to retrospective analyses, several studies were carried out to evaluate these mechanisms *in vitro* [38,40] or *in vivo* [40]; in some tests nicotine concentrations that exist in the blood of smokers were used [2].

The article focuses on nicotine as a factor able to trigger resistance of lung cancer to systemic therapy or radiotherapy. Well-defined mechanisms promoting survival and progression of lung cancer are described.

Clinical impact of nicotine

Although a number of studies have documented the poorer outcome in patients who continue smoking after cancer diagnosis, data about solely nicotine's influence on patients' survival are lacking.

The first *in vitro* and *in vivo* study that evaluated the direct effects of nicotine administration on cancer cell survival and the role of nicotine on response to radiotherapy (RT) or chemoradiotherapy (CRT) was published in 2012 by Warren et al. [40]. They demonstrated that nicotine increased the ability to survive of two cell lines (human H460 and A549 lung cancer cells) following exposure to RT *in vitro* (p < 0.05). In a mouse model, the administration of nicotine during RT or CRT increased xenograft regrowth (p < 0.05) as compared to RT or CRT alone. The observed effect seemed to occur through a decreased therapeutic response to radiation or chemotherapy rather than through increased proliferation after completion of treatment [40].

IMPACT ON DRUG METABOLISM

Cigarette smoking is known to alter the metabolism of several drugs by induction of cytochrome P450. The most significant effect is thought to be correlated with polycyclic aromatic hydrocarbons from tobacco smoke, which are responsible for the induction of key drug metabolizing enzymes such as CYP1A1, CYP1A2 and CYP2E1 [45]. This acceleration of metabolism in smokers
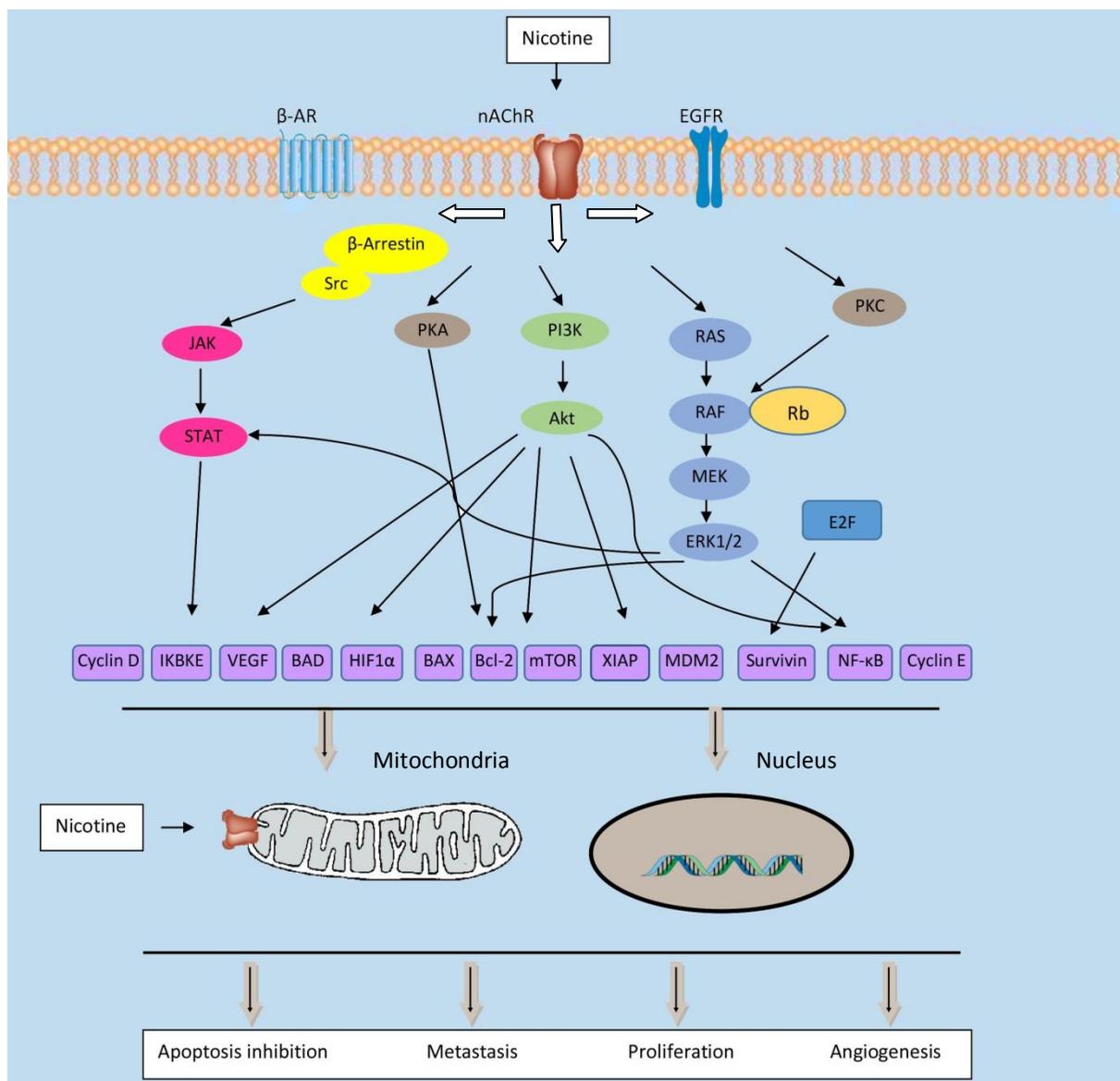
Fig. 1. Principal mechanisms of nicotine-induced proliferation, migration, angiogenesis and inhibition of apoptosis in lung cancer cells

affects the pharmacokinetics and/or pharmacodynamics of drugs commonly used in lung cancer treatment such as erlotinib, gemcitabine, and taxanes, resulting in higher drug clearance and lower toxicity [12,16,30]. Conversely, there are few data on the influence of nicotine on metabolism of chemotherapeutic agents. This main alkaloid in tobacco smoke is primarily metabolized in the liver by CYP2A6 and, to a lesser extent, by CYP2B6 and CYP2E1 [19]. The impact of nicotine on activity of CYP enzymes has been analyzed mainly in rats; therefore the effect on cytostatics metabolism in humans is still poorly defined.

Mechanisms of modification of response to treatment

The observations that smoking/nicotine may influence the response of lung cancer to treatment gave rise to

further research about the mechanism of this effect. Numerous studies have demonstrated that nicotine affects the results of treatment in many different ways. Figure 1 illustrates the most important intracellular pathways altered by nicotine.

RECEPTORS OF NICOTINE SIGNALING

Nicotinic acetylcholine receptors (nAChRs)

Nicotine has an ability to penetrate all tissues in the body. Its biological effects are mediated by nicotinic acetylcholine receptors (nAChRs) that are expressed on both normal and human lung cancer cells [36]. The receptors are ligand-gated ion channels that mediate signal transmission of endogenous acetylcholine and exogenous substances such as nicotine and tobacco-

specific nitrosamines (NNN, NNK). Because of higher receptor-binding affinity, nicotine can displace acetylcholine from nAChRs expressed in cancer cells, stimulate them and lead to the activation of several signaling pathways, such as increase of influx of Ca^{2+} and activation of calmodulin or various kinases (PI3K, Akt, MAPK, PKC); these actions result in a decrease of apoptosis, and an increase of proliferation, angiogenesis and the ability to form metastases [6,36,43,47]. Moreover, nicotine increases production and secretion of acetylcholine in lung cancer cells and upregulates nAChRs expression [5,22]. The forced acetylcholine-mediated pathway provides stimuli to lung cancer cells as it regulates multiple cellular functions, for example proliferation. Inhibition of this acetylcholine-mediated signaling by vesamicol (a vesicular acetylcholine transporter antagonist) causes apoptosis of nicotine-treated lung cancer cells, whereas it has no effect on EGF or IGF-II-induced proliferation. It was suggested that vesamicol suppressed Akt activation, which plays a vital role in the nAChR signaling pathway [22]. On the other hand, nicotine can stimulate cancer cell proliferation in spite of auto/paracrine acetylcholine deprivation [8].

β-adrenergic receptors (β-ARs)

There is some evidence that activation of β-ARs is also implicated in nicotine mitogenic signal transduction pathways in lung adenocarcinomas [31]. For example, in an *in vitro* study on pulmonary adenocarcinoma cells propranolol, a β-AR inhibitor, blocked nicotine-induced activation of ERK1/2 (a subset of the MAPK family), Akt, PKA, and Bad phosphorylation, therefore promoting apoptosis after treatment with cisplatin and etoposide [20]. This process can be at least in part caused by the release of noradrenaline by nicotine-stimulated NSCLC cells [1].

Indirect effect on epidermal growth factor receptors (EGFRs)

Cigarette smoke induces activation of EGFR and impairs receptor degradation. The abnormal stabilization of the activated receptor leads to uncontrolled cell growth and tumorigenesis. It changes the conformation of both WT and MT L858R *EGFR* at the level of the intracellular domain [21]. Cigarette smoke leads to a novel EGFR conformation that interacts strongly with c-Src, and its activity mediates cancer growth and is not inhibited by EGFR tyrosine kinase inhibitors (TKIs). Researchers showed in *in vitro* studies that under cigarette smoke exposure signals leading to cell survival are prolonged and TKIs cannot inhibit the cigarette smoke-induced activation of ERK 1/2 and Akt. Moreover, they observed that TKI-sensitive cells (NIH-3T3 and NSCLC cells harboring the TKI-sensitive EGFR mutation) become resistant to TKIs, which results in clonal growth of lung cancer even in the presence of TKIs [13]. This mechanism of resistance has been proposed to be associated with nicotine-induced nAChRs activation and cross-talk between EGFR and nAChR. In an *in vitro* study, nicotine increased expression and phosphorylation of EGFR and, simultaneously, Act and ERK phosphorylation [39].

There is other growing evidence of such interaction between nAChRs and growth factor receptors by molecular signaling cascades. It has been documented that combination of nicotine with EGF, IGF or VEGF increases lung cell and lung cancer cell proliferation more than each stimulant given alone [8].

ANTI-APOPTOTIC AND PRO-SURVIVAL EFFECT OF NICOTINE

Nicotine has been found to attenuate apoptosis caused by opioids, cisplatin, etoposide, paclitaxel, TNFα, RT, ultraviolet irradiation and hydrogen peroxide [9,24,25,36,40,41,46]. This protective effect was mediated by diverse intracellular pathways listed below.

PI3K/Akt pathway

The PI3K/Akt-related pathway plays a crucial role in development and progression of lung cancer and in mediating chemotherapy-induced apoptosis.

Data indicate that phosphoinositide 3-kinase is a potential mediator of nicotine's impact on the therapeutic response. In an *in vitro* study inhibition of PI3K prevented nicotine-mediated increase of cell survival following RT and induction of Akt, MMP-2 and HIF-1α [40].

Akt kinase was found to be constitutively active in NSCLC cells. Moreover, modulation of its activity alters cellular responsiveness to chemotherapy or radiation [3]. Nicotine (as well as other tobacco components, e.g. NNK) activates Akt in a dose-dependent and time-dependent manner. Thus, the process mediates the anti-apoptotic effect of nicotine on NSCLC cells. For example, pretreatment of A549 cells with an Akt inhibitor caused a slight reduction in chemotherapy-induced apoptosis and blocked the effects caused by nicotine in these cells [46]. Activated Akt mediates phosphorylation of downstream substrates such as pro-apoptotic *BCL2*-antagonist of cell death (BAD), pro-apoptotic protein BAX, up-regulation of anti-apoptotic XIAP (a protein that suppresses apoptosis by binding to caspases-3, -7, and -9) [9,20] and other anti-apoptotic and pro-proliferative factors mentioned below.

BAD

BAD is a pro-apoptotic protein that has been shown to interact with one or more of the death suppressor family members, such as Bcl-X_L. According to some studies, nicotine induces BAD phosphorylation and therefore prevents activation of mitochondrial-based cell death and enhances survival of NSCLC cells. This process may occur through several signaling pathways involving ERK1/2 (extracellular signal-regulated kinase), PI3K/AKT and PKA. Moreover, specific BAD expression knockdown enhances chemoresistance [20,46].

Survivin and XIAP

Survivin and XIAP are members of the inhibitor of apoptosis protein family (IAPs) that prevent activation of caspase-family proteases. In one study, exposure of NSCLC cells to nicotine inhibited apoptosis induced by cisplatin, paclitaxel and gemcitabine, and this was correlated with induction of survivin and XIAP. The effect was abrogated by depletion of IAP proteins with siRNA. Furthermore, the researchers revealed that nicotine mediates the recruitment of E2F1 (E2F transcription factor 1), which leads to dissociation of Rb (retinoblastoma tumor protein) from the survivin promoter. Additionally, ablation of E2F1 level inhibits the protective effect of nicotine on cisplatin-induced apoptosis in A549 cells [9].

MAPK

There are some indirect data that imply a critical role of the MAPK pathway in both cisplatin-induced apoptosis and in mediation of the anti-apoptotic effects of nicotine. Zhang et al. used small interfering RNA (siRNA) to differentiate the role of MAPK in this process in A549 cells [46]. The results suggest that MEK1 (MAP2K1) mediated the anti-apoptotic effects of nicotine, whereas MEK2 (MAP2K2) mediated the chemotherapy-induced apoptosis.

BCL2

BCL2 is a key regulator of programmed cell death and apoptosis that is expressed in SCLC and NSCLC cells. The protein regulates mitochondrial membrane permeability, and therefore interferes with the release of mitochondrial apoptotic factors. BCL2 contributes to a more chemoresistant phenotype of NSCLC, as sensitivity to cisplatin seems to be partially dependent on BCL2. Downregulation of this pro-survival factor sensitizes lung cancer cells to cisplatin, but interestingly not to docetaxel, which inactivates the BCL2 function [23]. It has been reported that nicotine up-regulates BCL2 in NSCLC cells, possibly by phosphorylation and higher expression through a pathway involving activation of PKC and/or the ERK1/2 kinases [18]. Furthermore, nicotine was found to induce BCL2/BAX heterodimerization (that blocks the death action of BAX), inhibit BCL2 ubiquitination, and prevent up-regulation and translocation of BAX to the mitochondria (by phosphorylation) in association with prolonged survival of lung cancer cell lines treated with cisplatin and etoposide [24,28,46].

NF-κβ

The resistance of NSCLC to cytotoxic drugs can be associated with NF-κB activity. Tsurutani et al. found that nicotine (in contrast to NNK) stimulates NF-κB activity in NSCLC cells, and therefore decreases apoptosis of NSCLC cells exposed to paclitaxel or etoposide. In this study, NSCLC cells expressing the NF-κB inhibitor IκBα were resistant to nicotine-mediated protection [36].

JAK/STAT pathway

It has been observed that nicotine and NNK also activate the JAK/STAT pathway in NSCLC cell lines and therefore alter expression of its target genes [14]. STAT3 is a transcription factor that regulates such intracellular processes as cell growth and apoptosis. In a study on NSCLC specimens Guo et al. observed co-expression of pSTAT3 and the product, IKBKE, of one of its target genes (*IKBKE* is an oncogene) and the association of elevated IKBKE with patients' smoking history. Furthermore, they found that depletion of IKBKE sensitizes NSCLC cells (treated with cisplatin, gemcitabine and doxorubicin) to chemotherapy-induced apoptosis and that, conversely, enforcing its expression induces chemoresistance [14].

Proliferation effect on lung cancer cells

Different mechanisms of nicotine-induced cell proliferation involve recruitment of β-arrestin and Src to the nAChR, which results in Src activation. This leads to the activation of MAPK, Raf-1 (which interacts with Rb), cyclin E and D. Since Raf-1 binds to Rb, it causes Rb inactivation and thereby enhances recruitment of E2F transcription factor 1 (E2F1) and Raf-1 to proliferative promoters. In one trial nicotine stimulation resulted in the dissociation of Rb, p107, p130 and repressive E2F transcription factors (E2F4 and E2F5), whereas it increased the binding of the proliferative E2Fs (E2F1–3) to mitogenic promoters [10].

Activation of the PI3K/Akt/mTOR pathway and correlation between MDM2 and p53 correlate with cellular proliferation in multiple cancers. Nicotine has a documented effect on phosphorylation of such Akt downstream substrates as mTOR and MDM2 in lung cancer cell lines and therefore promotes proliferation and cell survival (not shown for NNK) [36].

Other mechanisms of induction of resistance
Interaction with mitochondria

Nicotine can act through interactions with mitochondria. The mitochondrial death pathway is one of the most important ways by which cytostatic drugs or radiotherapy induce apoptosis and death of cancer cells. Zhang et al. proved that nicotine inhibits apoptosis induced by cisplatin and etoposide via its impact on this pathway [46]. In a study conducted on NSCLC cell lines, nicotine exposure prevented chemotherapy-induced reduction of potential in mitochondrial membrane and blocked caspase-9 activation and translocation of BAX to the mitochondria in NSCLC cells. Additionally, A549 lung cancer cells with a lack of mitochondrial DNA were partially resistant to chemotherapy-induced apoptosis; however, the anti-apoptotic effect of nicotine was blocked as well. Moreover, the effect was also blocked by an inhibitor of the mitochondrial ion channel. The authors concluded that intact mitochondria and mitochondrial signaling are essential in nicotine's cell-pro-

tective effect [46]. Recent data revealed that nicotine can penetrate cancer cells and act by stimulation of mitochondrial nAChR subunits whose expression increases during malignant transformation. This activation inhibits intrinsic apoptosis by prevention of opening of mitochondrial permeability transition pores [8].

Influence on DNA synthesis

An anti-apoptotic effect of nicotine may in part occur through augmented DNA synthesis. In the study mentioned above, lung cancer cells exposed to nicotine with or without cytostatics (cisplatin, etoposide) modestly increased the uptake of ^{3}H-thymidine [46]. The phenomenon is probably associated with the influence of nicotine on mitochondria.

On the other hand, ^{3}H-thymidine uptake was also increased in human lung epithelial cells exposed to nicotine or nicotine and γ-radiation [29]. In this study nicotine promoted some of the irradiated cells to divide. Furthermore, it was found to block activation of G1/S checkpoints mediated by γ-radiation, probably by up-regulation of cyclins D1 and A and attenuation of Chk2. Conversely, nicotine does not compromise the nocodazole-mediated G2/M phase checkpoint [29]. The finding that nicotine disrupts radiation-mediated DNA damage may explain, at least partially, the mechanism of development of resistance to radiation therapy in smoking patients.

Interaction with tobacco-derived nitrosamines

Nitrosamines such as NNK and NNN are well-defined carcinogens with additional activity of cell growth stimulants by association with nAChRs. It was demonstrated that nicotine modulates NNK-mediated signaling and therefore persistently increases lung cancer cells' resistance to cisplatin [27].

Downregulation of retinoic acid receptor (RAR)

The approach to prevent lung cancer development in smoking humans by vitamin A supplementation that was based on animal and epidemiological studies brought disappointing results. The growth inhibitory effect of all trans-retinoic acid is mediated by retinoid acid receptors, but nicotine suppressed the effect by inhibiting RARβ expression [7].

Metastasis and invasion promotion

In 2006 Xu et al. discovered that nicotine potently induces phosphorylation of both μ- and m-calpains via activation of protein kinase Ciota (PKCiota), which is associated with accelerated migration and invasion of human lung cancer cells [43].

Similar results were obtained in 2007 by Zhang et al., who showed that exposure to nicotine significantly promoted invasion of A549 cells (10-fold) compared with

controls. This process could be, in part, dependent on HIF-1α expression [47].

Epithelial to mesenchymal transition (EMT)

Long-term exposure to nicotine downregulates E-cadherin and β -catenin with concomitant increase of expression of fibronectin and vimentin. Thereby it enhances adherence-independent proliferation of tumor cell lines and allows the cells to grow robustly independently of a cell substratum, which causes cancer progression [11].

Another protein involved in epithelial to mesenchymal transition, cancer cell proliferation, drug resistance and invasion induced by nicotine is periostin. It is a secreted adhesion-related protein abnormally highly expressed in lung cancer that correlates with angiogenesis, invasion and metastasis. Periostin is one of the targets upregulated by nicotine in lung cancer cells. Data from the Wu et al. *in vitro* study on A549 NSCLC cells with a silenced periostin gene (using small interfering RNA (siRNA)) revealed that A549 cells showed reduced cell proliferation, elevated sensitivity to chemotherapy with cisplatin, decreased cell invasion and Snail expression [42].

Angiogenesis

Many researchers have reported that nicotine has some proangiogenic activity [33,34]. Others suggest that nicotine can mimic the effects of hypoxia, and therefore is responsible for the high metastatic potential of lung cancer [15].

In 2007 Zhang et al. reported that nicotine significantly stimulates hypoxia-inducible factor 1-alpha (HIF-1α) protein accumulation and VEGF expression (in part by the influence of HIF-1α) in human NSCLC [47]. Moreover, they observed that HIF-1α contributes to nicotine-enhanced *in vitro* tumor angiogenesis, migration and invasion. Similar results were obtained by Warren et al. in a study on lung cancer xenografts – nicotine increased expression of HIF-1α, but this effect was transient and reversible (2-3 days after nicotine removal) [40]. Moreover, there was no correlation between HIF-1α expression and expression of carbonic anhydrase (a clinical marker of hypoxia). *In vitro* experiments aimed at assessing whether nicotine exposure enhances tumor angiogenesis were also conducted by Heeschen et. al [17]. In a Lewis lung cancer model they observed that nicotine-treated mice showed significantly faster tumor growth (p < 0.01) and had significantly higher capillary density in the tumors (P < 0.001) than the vehicle-treated group. In another experiment they found that VEGF concentration was significantly higher in the nicotine group compared with controls (p < 0.001). The influence of nicotine on angiogenesis has also been suggested by the results of an experiment conducted by Zhu et al. [48]. They found that second-hand smoke significantly increased

tumor size, weight, capillary density, VEGF and MCP-1 levels, and circulating endothelial progenitor cells (EPC). This effect was suppressed by mecamylamine (an nAChR inhibitor), which reduced VEGF and EPC levels.

Guo et al. investigated the mechanism of nicotine-induced HIF-1α up-regulation and found that it is not connected with higher synthesis (no change in mRNA level) but probably is mediated by mitochondrial ROS that stabilize HIF-1α [15]. Moreover, it was demonstrated that nicotine induced up-regulation of VEGF and Glut-1 in a HIF-1α dependent manner. They also found that accumulation of HIF-1α is caused by stimulation of nAChRs and involves activation of Akt and MAPK pathways.

Despite the mentioned pro-angiogenic effect, up-regulation of HIF-1α was found to increase resistance of breast cancer cells to apoptosis (induced by docetaxel) by activation of survivin gene transcription [32].

Conclusion

Nicotine, a cigarette smoke component, modulates intrinsic cellular signaling pathways at multiple levels. Its effects are mediated mainly by neuronal acetylcholine receptors, but it is reported that nicotine can act through β-adrenergic receptors or directly through the interactions with mitochondria. Due to modulation of activity of many cellular proteins, nicotine induces cell survival, proliferation and angiogenesis, and promotes invasion and metastasis. Many of these processes may influence the effectiveness of anti-cancer treatment (chemotherapy, radiotherapy or targeted therapy). There is some evidence that patients who continue to smoke have worse survival than those who give up smoking before treatment; it is also possible that nicotine supplementation for smoking cessation might reduce the response to anti-cancer agents. Although nicotine is not the main component of tobacco smoke, there is growing evidence of its detrimental activity. Unfortunately, the most important proofs of nicotine's influence on treatment efficiency are based on *in vitro* studies which do not represent clinical conditions. Further evaluation of its anti-apoptotic effects may facilitate the improvement of therapeutic options for those patients with NSCLC who were or still are exposed to nicotine.

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