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## **Metabolic reprogramming by tobacco smoke-derived nitrosamines (TSNAs) in cancer.**

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**Summary:** Metabolic reprogramming is a hallmark of cancer and the link between oncogenes activation, tumor suppressors inactivation and bioenergetics modulation is well established. However, numerous carcinogenic environmental factors are responsible for early cancer initiation and their impact of metabolic reprogramming just starts to be deciphered. For instance, it was recently shown that UVB irradiation triggers metabolic reprogramming at the pre-cancer stage with implication for skin cancer detection and therapy. These observations foster the need to study the early changes in tissue metabolism following exposure to other carcinogenic events. According to the International Agency for Research on Cancer (IARC), tobacco smoke is a major class I-carcinogenic environmental factor that contains different carcinogens, but little is known on the impact of tobacco smoke on tissue metabolism and its participation to cancer initiation. In particular, tobacco-smoke derived nitrosamines (TSNAs) play a central role in tobacco-smoke mediated cancer initiation. Here we describe the recent advances that have led to a new hypothesis regarding the link between nitrosamines signaling and metabolic reprogramming in cancer.

## Abbreviation List

3PG: 3-phosphoglycerate  
4E-BP1: 4E-binding protein 1  
8-OHdG: 8-hydroxy-2'-deoxyguanosine  
AA: arachidonic acid  
ACC1: acetyl-CoA carboxylase-1  
AMPK: Adenosine monophosphate-activated protein kinase  
AP1: activator protein 1  
AREs: antioxidant response elements  
ASK1: Apoptosis signal-regulating kinase 1  
ATF-1: activating transcription factor-1  
 $\beta$ -AR :  $\beta$ -adrenergic receptor  
cAMP: cyclic AMP  
CDKN2A:  
CIN : Chromosome instability  
CoA: acetyl-coenzyme A  
COPD: chronic obstructive pulmonary disease  
COX : Cyclooxygenase  
CREB: cAMP response element binding protein  
CREM: cAMP response element modulator  
EGF: epidermal growth factor  
EGFR: epidermal growth factor receptor  
ETC: electron transport chain  
F6P: fructose 6-phosphate  
FAKHR or FOXO: forkhead transcription factors  
FAO: fatty acid oxidation  
FAS: fatty acid synthase  
FH: fumarate hydratase  
GPCR G: protein-coupled receptors  
GSK-3: glycogen synthase kinase-3  
H<sub>2</sub>O<sub>2</sub>: Hydrogen peroxide  
HIF1 $\alpha$ : hypoxia inducible factor 1 $\alpha$   
HMOX1: haem oxygenase 1  
HREs: hypoxia response elements  
IARC: International Agency for Research on Cancer  
IDH1: isocitrate dehydrogenase 1  
*iso*-NNAC: 4-(methylnitrosamino)-4-(3-pyridyl) butanoic acid  
*iso*-NNAL :4-(methylnitrosamino)-4-(3-pyridyl)-1-butanol  
JAK: Janus-activated kinase  
JNKs: c-Jun N-terminal kinases  
KEAP1: Kelch-like ECH-associated protein 1  
*LDHA*: lactate dehydrogenase A  
LKB1: liver kinase B1  
LOX: lipoxygenase  
MAPKKK : MAP kinase kinase kinase  
MEKs: mitogen-activated protein kinase kinases  
mtDNA : mitochondrial DNA ()  
mTORC1: mechanistic target of rapamycin complex 1  
mtPTP: mitochondrial permeability transition pore  
mtROS: mitochondrial ROS  
N7-meG: N7-methylguanine  
NAB: *N*'-nitrosoanabasine

nAChR: nicotinic acetylcholine receptor  
NAT *N'*-nitrosoanatabine  
nDNA: nuclear DNA  
NF- $\kappa$ B: nuclear factor  $\kappa$ B  
NNA: 4-(methylnitrosamino)-4-(3-pyridyl) butanal  
NNAL: 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol  
NNK: 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone  
NNN: *N'*-nitrososnormicotine  
NRF2: Nuclear factor (erythroid-derived 2)-like 2  
NF2: neurofibromin 2  
NSCLC: non-small cell lung cancer  
O<sub>2</sub><sup>-</sup>: Superoxide anion  
O<sup>4</sup>-meT: O<sup>4</sup>-methylthymine  
O<sup>6</sup>-meG: O<sup>6</sup>-methylguanine  
OAA: oxaloacetate  
OH·: Hydroxyl radical  
OXPHOS: oxidative phosphorylation  
P450s: Cytochrome P450 enzymes  
PDK: pyruvate dehydrogenase kinase  
PGC-1 $\alpha$ : nuclear transcriptional coactivator peroxisome proliferator-activated receptor  $\gamma$  coactivator 1- $\alpha$   
PHD: prolyl hydroxylase  
PI3K: phosphatidylinositol 3-kinase  
PIP3: phosphatidylinositol 3,4,5-triphosphate  
PKA: protein kinase A  
PKB: protein kinase B  
PKC: protein kinase C  
PKM2: pyruvate kinase M2 isoform  
PLA2: phospholipase-A2  
POB: pyridyloxobutylations  
PPP: pentose phosphate pathway  
PRAS40: proline-rich Akt substrate 40 kDa  
PRXs: peroxiredoxins  
R5P: ribose 5-phosphate  
Raptor: regulatory associated protein of mTOR  
Rb: retinoblastoma  
ROS: Reactive oxygen species  
RTK: receptor tyrosine kinase  
RYO: roll-your-own  
S6K1: S6 kinase 1  
SDH: succinate dehydrogenase  
SHMT: serine hydroxymethyltransferase  
SREBP-1: sterol regulatory element-binding protein 1  
STAT3: signal transducer and activator of transcription 3  
TFAM: mitochondrial transcription factor A  
TSC2: tuberous sclerosis 2  
TSC2tuberous sclerosis 2  
TSNAs: Tobacco-specific nitrosamines  
TxA2: thromboxane A<sub>2</sub>

## Introduction

Cigarette smoke is the predominant form of tobacco consumption, and it is the largest preventable cause of cancer, causing approximately 30% of all cancer deaths (1-3). Tobacco smoke contains approximately 7000 different types of molecules. Among these compounds, more than 60 are known carcinogens, and several are tumor promoters or co-carcinogens (1). Carcinogenesis is a continuous process initiated by genetic, epigenetic and metabolic alteration, followed by other factors that stimulate growth, survival and spreading of the cell. The carcinogens contained in tobacco smoke belong to various chemical classes, including polycyclic aromatic hydrocarbons (PAHs) (such as benzo[*a*]pyrene), tobacco-specific nitrosamines (such as NNK and NNN), aromatic amines (such as 4-aminobiphenyl), aldehydes, phenols, volatile hydrocarbons, nitro compounds, and other organic and inorganic compounds (2). Tobacco can be used in different forms: smoking (roll-your-own (RYO) or manufactured cigarettes, cigars that are made of air-cured and fermented tobaccos with a tobacco-leaf wrapper, water pipe, pipe, etc.) or smokeless (moist and dry snuff, chewing tobacco). The main focus of this review is on one of the most carcinogenic components of tobacco smoke, nitrosamines. Tobacco-specific nitrosamines (TSNAs) only exist in tobacco products. During fresh green tobacco air curing, storage and fermentation, nicotine and minor tobacco alkaloids can be converted to TSNAs through nitrosation (6-8). This group of nitrosamines consists of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), 4-(methylnitrosamino)-4-(3-pyridyl) butanal (NNA), *N'*-nitrosoanatabine (NNN), *N'*-nitrosoanabasine (NAB), *N'*-nitrosoanatabine (NAT), 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL), 4-(methylnitrosamino)-4-(3-pyridyl)-1-butanol (*iso*-NNAL), and 4-(methylnitrosamino)-4-(3-pyridyl) butanoic acid (*iso*-NNAC) (3), among which NNN and NNK, due to sufficient evidence in experimental animals, are classified as human carcinogens (Group 1) by the International Agency for Research on Cancer (IARC) (4). It is well documented that NNK and NNN can induce several malignancies, including those of the lung, esophageal, liver, pancreatic and nasal cavity (11-17). NNK causes lung tumors in all tested species, and its activity in rats is particularly high (5). To study the role of cigarette smoke in health *in vitro*, two different methods are currently used: acute exposure and chronic exposure. Acute exposure can provide general insight about the alterations related to tobacco smoke. Data from these kinds of short-term studies can reveal effects that may constitute the underlying causal chain leading to the ultimate chronic effects. For instance, it has been shown that cigarette smoke can induce apoptosis in cells when applied at higher concentrations (6-8). Moreover, it has been reported that acute smoking causes pro-inflammatory states in the lung that can lead to inflammatory diseases such as COPD (chronic

obstructive pulmonary disease) and asthma and promote tumorigenesis. In general, acute exposure studies could demonstrate cellular changes similar to cancerous cells (9). Although acute studies help to understand numerous details about the effects of cigarette smoking, it is still not possible to understand the exact mechanism underlying tobacco smoke as studying some effects (such as cellular transformation) is only feasible with chronic exposure. As a result, to study the molecular alterations, long-term studies are recommended. Until now, several chronic exposure studies have been conducted in vitro, providing valuable information about metabolic changes due to chronic tobacco smoke (10–14); however, additional chronic studies are needed to better understand the detailed cellular mechanism of tobacco smoke.

The levels of TSNA in smokeless tobacco depends on the preparation used (15) and the variations in TSNA content of cigarette tobacco are also appreciable. For instance, Greek and Turkish types of tobacco are generally characterized by low TSNA contents. Conversely, French Gauloises had 8.6  $\mu\text{g/g}$  of TSNA, whereas the TSNA concentrations in US and German brands range from 1.6–5.5  $\mu\text{g/g}$  (15). Numerous lines of evidence have established that NNK and NNN induce carcinogenesis by causing DNA adductions and mutations as well as promoting tumor growth through receptor-mediated effects, which are facilitated by associated receptors, such as nicotinic acetylcholine receptor (nAChR) and  $\beta$ -adrenergic receptors ( $\beta$ -AR)(20,21). However, additional cancer-driving mechanisms could be stimulated by nitrosamines, and little attention has been given to the global impact of TSNA on the emerging cancer hallmarks. The hallmarks of cancer include the sustained proliferative signaling, evasion of growth suppressors, resistance to cell death, enabling of replicative immortality, induction of angiogenesis, activation of invasion and metastasis, reprogramming of energy metabolism, genome instability and mutation, avoidance of immune destruction and promotion of tumor inflammation. In particular, one emergent hallmark of cancer is metabolic reprogramming, and our review will discuss the link between TSNA and **the changes in tumor bioenergetics associated with carcinogenesis. In particular, the main signaling pathways activated by TSNA, namely  $\beta$ -AR and nAChR signaling, have also been involved in the regulation of energy metabolism but only few studies have considered the direct bioenergetic impact of TSNA on pre-cancer or cancer cells. The modulation of mitochondrial biology by TSNA is another point of discussion as changes in organelle biogenesis were described upon activation of Beta-adrenergic signaling. Lastly, TSNA have been shown to activate different proto-oncogenes that could consecutively modulate energy metabolism. These different aspects are covered in what follows.**

### **TSNA-induced carcinogenic genomic alterations**

Most carcinogens in tobacco products undergo metabolic activation before they are able to react with DNA. In this process, an unreactive carcinogen is converted to a form that is able to bind to DNA. There are also some carcinogens that can act without any activation process (16). Cytochrome P450 enzymes (P450s) generally initiate this metabolic activation for foreign compounds, including natural products, pharmaceuticals, and carcinogens. P450s catalyze reactions in which produced metabolites are either excreted in a detoxified form or readily undergo further detoxification reactions. As long as this detoxification process works efficiently, cells are protected. However, some metabolites resulting from this carcinogen-induced metabolic activation are electrophilic (have an electron-deficient center) and can react with DNA. The reaction of these metabolites with DNA can lead to the generation of DNA adducts, which are generally regarded as intermediaries in the mutagenesis process. DNA adducts can be removed by the cellular repair system, but some can also escape from this system and subsequently initiate the carcinogenic process (3,17). It has been reported that NNK can be activated through different reactions by cytochrome P450s:  $\alpha$ -hydroxylation, pyridine oxidation, and carbonyl reduction (18). Such activation generates the toxic methylating agent methanediazohydroxide which further reacts with DNA to produce N7-methylguanine (N7-meG), O<sup>6</sup>-methylguanine (O<sup>6</sup>-meG) and O<sup>4</sup>-methylthymine (O<sup>4</sup>-meT). During the activation process, the carbonyl group in NNK is also reduced to produce NNAL that will generate glucuronide or undergo methylene hydroxylation (Figure 1). The excretion of NNAL and its glucuronide represents a useful index of exposure in tobacco users (19,20). There is also another pathway in which 4-(3-pyridyl)-4-oxobutane-1-diazohydroxide will form, which leads to the introduction of pyridyloxobutyl (POB) adducts in DNA (21–23). Oxidative metabolism of NNN generates the same reactive diazohydroxide as obtained upon  $\alpha$ -hydroxylation of the terminal methyl group of NNK, thereby introducing POB in proteins and DNA. NNN exhibits a complex pattern of metabolites in urine (24,25). Three important types of DNA damage can be caused by NNK and NNN: nucleotide methylation, pyridyloxobutylations as well as pyridylhydroxybutylation (Figure 1). As mentioned above, DNA phosphate POB adducts are also caused by these TSNA (26). The N7-meG is the predominant adduct found in target tissues induced by NNK, followed by O<sup>6</sup>-meG, whereas very low levels of O<sup>4</sup>-meT are present (15). It has been demonstrated in the lung that by  $\alpha$ -hydroxylation, NNK can transform into either a methylating agent or a pyridyloxobutylating agent. The methylation pathway, which is critical for NNK-induced lung tumorigenesis (in A/J mice), generates methyl DNA adducts such N7-meG and O<sup>6</sup>-meG (37-40). The pyridyloxobutylating agent produce relatively bulky DNA adducts (27). It has been shown that NNK pyridyloxobutylation is a potential initiating event of CIN in lung carcinogenesis

(28). Chromosome instability (CIN), which is an abnormal increase in the rate of change in chromosome number and structure, is assumed to be a common genomic instability in tumor evolution (29). It has been documented that NNK is also able to induce extensive genome-wide changes in DNA copy number (or in general chromosomal alterations), indicative of CIN (28). Gross chromosomal alterations have been repeatedly observed in NNK-induced mouse lung adenocarcinomas (28,30). In general, the majority of research reports that most DNA adducts derived from cigarette-smoke carcinogens, including nitrosamines, cause miscoding permanent mutations (most frequently G–T and G–A) (31) that can occur in crucial regions of oncogenes or tumor-suppressor genes. These mutations can lead to cancer development by creating disruptions in cell-cycle checkpoints, chromosomal instability and other changes (32). Candidate NNK-induced genes with roles in lung carcinogenesis are p53 and retinoblastoma (*Rb*) tumor suppressor genes, and KRAS oncogene (28). NNK-mediated activation of the C-Myc oncogene could also participate to CIN. Partial evidences demonstrated that, on the one hand, NNK could activate C-Myc through Thr<sub>58</sub> and Ser<sub>62</sub> phosphorylation in a ERK1/2 and PKC-alpha dependent manner (33), and on the other hand, that Myc activation could generate CIN (34). Yet, C-Myc activation is not a major contributor to lung carcinogenesis that depends more on KRAS, suggesting that NNK-mediated KRAS activation could contribute to CIN in this type of cancer (35). Lastly, gene silencing by promoter hypermethylation is an emerging mechanism that might also be important in tobacco carcinogenesis (36). To conclude on the TSNA-induced carcinogenic genomic alterations, one should also consider the importance of the cumulated dose of tobacco-smoke exposure in this phenomenon. Comparison of the genomics and epigenomics alterations in lung tumors from smokers and from never smokers revealed important differences in the mutational status of KRAS and STK11 (LKB1) that showed a higher level of alteration in smokers (37). Given the role of LKB1 in the control of energy metabolism through the AMPK pathway (38), bioenergetic differences could be expected in lung tumors of smokers as compared to never smokers. Accordingly, the gene expression profiling of lung and plasma from mice exposed to tobacco smoke during seven days to nine month revealed significant changes in the expression of AMPK-target genes as oxidative phosphorylation enzymes (39), but the underpinning signaling mechanisms governing these bioenergetic alterations still remain unknown.

### **Nitrosamine receptors and signaling**

Nicotine and its oncogenic derivatives NNK and NNN are thought to promote tumor progression by binding to and activating the two cell surface receptors nicotinic nAChR and



$\beta$ -AR. This activation can lead to the stimulation of multiple downstream tumor-promoting signal transduction pathways (40) discussed below. **The two main branches of TSNAs signaling, namely  $\beta$ -AR and nAChR intertwined pathways, play a role in the reprogramming of energy metabolism and could thus be involved in the bioenergetic reprogramming of pre-cancer and cancer cells, as discussed below. At first, we provide a rapid description of  $\beta$ -AR and nAChR signaling pathways.**

### **Adrenergic receptors (AR)**

Adrenergic receptors (AR) are a family of G protein-coupled receptors (GPCR). These receptors can initiate multiple signaling cascades and therefore lead to numerous pathological conditions (41). There are two classes of adrenergic receptors, the  $\alpha$ -adrenergic and the  $\beta$ -adrenergic families (42). It has been demonstrated that  $\beta$ -ARs may regulate different processes of cancer initiation and progression, including the adenylyl cyclase/cAMP/PKA/CREB pathway (**Figure 2 (ex Fig.3)**), which transactivates the epidermal growth factor receptor (EGFR) pathway, the SRC/STAT pathway as well as the arachidonic acid (AA) cascade (43). The physiological agonists for  $\beta$ -ARs are catecholamine stress neurotransmitters noradrenaline and adrenaline (44), and their physiological inhibitor is a neurotransmitter GABA that can block the activation of adenylyl cyclase. NNK structurally resembles classical  $\beta$ -AR agonists (45), and consequently, it can activate downstream signaling cascades of this receptor. There is a body of evidence suggesting that NNK can induce cancer through  $\beta$ -AR activation, for which there is evidence from lung and pancreatic adenocarcinoma (46). **As discussed below,  $\beta$ -AR activation can also modulate mitochondrial biology and energy metabolism, which might participate to the bioenergetic reprogramming of lung exposed to cigarette smoke (39). The TSNAs-  $\beta$ -AR signaling pathway can be divided in three branches detailed below: (i) TSNAs-  $\beta$ -AR-PLA2, (ii) TSNAs-  $\beta$ -AR-ERK1/2 and (iii) TSNAs-  $\beta$ -AR-EGFR.**

At first, NNK can bind to  $\beta$ -AR on pulmonary epithelial cells and initiate proliferation cell signaling pathways, which leads to lung adenocarcinoma (47). After NNK binding to  $\beta$ -AR, GPCR triggers the activation of adenylyl cyclase and cyclic AMP (cAMP). cAMP itself activates protein kinase A (PKA), which subsequently activates phospholipase-A2 (PLA2) and defines the **TSNAs- $\beta$ -AR-PLA2** branch. PKA can also stimulate RAP-1 to define the **TSNAs- $\beta$ -AR-ERK1/2** branch. Then, cAMP can also activate EGFR which define the **TSNAs- $\beta$ -AR-EGFR** branch.

In the **TSNAs- $\beta$ -AR-PLA2** branch, arachidonic acid (AA) acts as a second messenger (**Figure 2**) to activate **mitogen-activated protein kinases and protein kinase C, which leads to**

increased DNA synthesis and cell proliferation in adenocarcinoma (47). Cyclooxygenase (COX) and lipoxygenase (LOX) are the two significant metabolic enzymes in AA metabolism (48) that have been shown to be overexpressed by pulmonary adenocarcinomas and pancreatic adenocarcinoma cells (49–51). In response to NNK, several products downstream of the COX pathway are produced, among which thromboxane A<sub>2</sub> (TxA<sub>2</sub>) has a significant role in cancer development and metastasis (52,53). In lung cancer cells, NNK leads to higher expression of thromboxane synthase TxAS, which facilitates TxA<sub>2</sub> synthesis from PGH<sub>2</sub> (prostaglandin H<sub>2</sub>) and leads to TxA<sub>2</sub> receptor activation (54). It has been demonstrated that overexpression of TxAS and TxA<sub>2</sub> receptor has a potential role in tumorigenesis and promotion of the progression of many types of cancers (52,55).

Regarding the TSNAs-β-AR-ERK1/2 branch, the NNK-mediated stimulation of β-AR signaling activates the cytoplasmic serine/threonine kinase effector B-RAF, which further activates signal-regulated kinase 1 and 2 (ERK1 and ERK2) (Figure 2) (56). The main genetic effectors of the TSNAs- β-AR signaling pathways include transcription factors as cAMP response element binding protein (CREB), cAMP response element modulator (CREM), activating transcription factor-1 (ATF-1), activator protein 1 (AP1) and the nuclear factor κB (NF-κB)(57). Those effectors alter cell proliferation, differentiation, survival, energy metabolism (new Figure 3) and consequently the promotion of oncogenesis (58).

Lastly, the TSNAs-β-AR-EGFR involves the transactivation of the EGFR pathway and downstream recruitment of the SRC kinase (59–63) that promotes activation of Ras oncogenic proteins. RAS, as a signal transducer, stimulates RAF and consequently mitogen-activated protein kinase kinases (MEKs), ERK1/2 and ribosomal protein S6 kinases (RSKs). These factors activate transcription factors such as AP1 and Myc, which regulate cell proliferation (46). Additionally, SRC can activate K<sup>+</sup> channels, which can stimulate the AA pathway (64). It has been reported that through this pathway, NNK regulates the growth and development of lung adenocarcinomas (65). In addition to RAF, RAS can also interact with phosphatidylinositol 3-kinase (PI3K), which is important in various cellular processes (Figure 2). Upon activation of PI3K, a secondary messenger phosphatidylinositol 3,4,5-triphosphate (PIP3) is generated, which further phosphorylates and activates serine/threonine kinase Akt or protein kinase B (PKB). The PI3K/Akt pathway is a critical pathway in cancer, as it is a regulator of key cellular processes that contribute to tumorigenesis, tumor growth and therapeutic resistance. As an example, it has been documented that NNK can, in this way, lead to the phosphorylation of the Bad/Bcl2 antagonist, the dissociation of which hampers the pro-apoptotic activity of Bad and consequently promotes cell survival and chemoresistance (33). The TSNAs-β-AR-EGFR branch includes activation of RAS (Figure 2) and involves

stimulation of signal transducer and activator of transcription 3 (STAT3), a transcription factor involved in lung cancer cells proliferation, metastasis and bioenergetics (66–68). Accordingly, the STAT3 target IKBKE, a serine/threonine kinase, is overexpressed in response to NNK and promotes proliferation (66). These findings might suggest that activation of beta-adrenergic signaling by NNK could mediate bioenergetic changes through STAT3 and further molecular investigation will be required to verify this hypothesis.

### **Nicotinic acetylcholine receptors (nAChRs)**

nAChRs consist of five subunits with homo- or heteropentamers that form ligand-gated ion channels located in the plasma membrane of mammalian cells (**Figure 3**). Initially, these receptors were identified in the nervous system, at the neuromuscular junction, and classified into neuronal or muscle nAChRs (108-111). The neuronal nAChRs can be homomeric (composed of five identical  $\alpha 7$ ,  $\alpha 8$  or  $\alpha 9$  subunits) or heteromeric (composed of combinations of  $\alpha 2$ – $\alpha 6$  or  $\alpha 10$  subunits and  $\beta 2$ – $\beta 4$  subunits), whereas muscle nAChRs may comprise combinations of  $\alpha 1$  subunits with  $\beta 1$ ,  $\gamma$ ,  $\delta$  or  $\epsilon$  subunits (70). Binding of agonist to nAChRs leads to the activation of these receptors, which is followed by ion influxes or recruitment of  $\beta$ -arrestin to the receptor to stimulate a number of intracellular signaling pathways (71,72). Acetylcholine is the initial agonist for these receptors, but nicotine and TSNAs can serve as an agonist for nAChRs, especially the  $\alpha 7$  subtype (73). The affinity of NNK for  $\alpha 7$ nAChR was found to be 1,300 fold higher than that of nicotine, whereas the affinity of NNN for heteromeric  $\alpha$ - $\beta$ nAChRs was 5,000 fold higher than that of nicotine (74,75). This higher affinity implies that NNK and NNN can win in the competition for binding to nAChRs. Therefore, many cardiovascular, neuropsychological and cancer-stimulating effects currently attributed to nicotine are probably caused by these nitrosamines (71). It has been shown that the binding of NNK to  $\alpha 7$ nAChR in lung cells lead to activation of voltage-gated calcium ( $\text{Ca}^{2+}$ ) channels and causes an influx of  $\text{Ca}^{2+}$  and consequently membrane depolarization (76). Following receptor activation and ion flux, three main sub-pathways have been described: *i*) nAChr-PKC-Erk1/2, *ii*) nAChr-VEGF and *iii*) nAChr-EGFR-RAS/PI3k. These sub-pathways are further discussed below. Please note the redundancy with the sub-pathways described above for the beta-adrenergic signaling also activated by TSNAs.

Regarding the nAChr-PKC-Erk1/2 branch, PKC activation leads to stimulation of the serine/threonine-protein kinase RAF (**Figure 3**). This activation initiates the MEK/ERK cascade as well as the downstream activation of number of transcription factors such as FOS, JUN and Myc, leading to proliferation in lung cancer (33). In this sub-pathway, NNK induces the phosphorylation of cellular proteases ( $\mu$ - and m-calpain) and leads to the migration and

invasion of lung cancer cells (77). Additionally, contactin 1, which is a neural cell adhesion molecule located in the plasma membrane, can be upregulated by NNK through the ERK pathway. Contactin 1, for which a role has been demonstrated in the development of various types of cancer including lung adenocarcinoma, is able to interact with several other membrane proteins or the extracellular matrix to activate downstream signaling pathways (78,79). The role of this molecule in lung cancer cell invasion and metastasis via the VEGF-C/Flt-4-mediated SRC-p38-C/EBP pathway has been confirmed (80–82). In addition, contactin 1 can prevent PHLPP2-mediated Akt dephosphorylation, which leads to Akt pathway activation (83). Additionally, nAChRs activation can activate the SRC kinase that leads in turn to RAF kinase stimulation and induction of the Rb/E2F1 axis which regulates proliferative genes as Cdc25A and Cdc6 (72).

The **nAChr-VEGF sub-pathway involves** HIF-1 $\alpha$  activation and plays a role in nicotine-induced tumor cell proliferation (84). In vitro studies performed on human lung cancer cell lines demonstrated that nicotine induces Hypoxia-Inducible Factor-1 $\alpha$  Expression through the nicotinic acetylcholine receptor with implication for lung cancer cell migration, and invasion (85).

Lastly, the **nAChr-EGFR-RAS/PI3k sub-pathway** involves the NNK-mediated stimulation of PI3K and Akt kinases (**Figure 3**) that leads to the activation of numerous downstream effectors: (a) forkhead transcription factors (FAKHR or FOXO), which have a wide range of roles in physiological processes, including cell cycle arrest, apoptosis, angiogenesis, stress resistance, energy metabolism, and stem cell differentiation (86), (b) serine/threonine kinase glycogen synthase kinase-3 (GSK-3), which can regulate glycogen synthesis, (c) tuberous sclerosis complex 2 (TSC2) or tuberin, which can control mechanistic target of rapamycin (mTOR) signaling pathway, (d) Apoptosis signal-regulating kinase 1 (ASK1) or MAP3K5, a member of the mitogen-activated protein kinase kinase kinase that activates downstream MAPKs, c-Jun N-terminal kinases (JNKs) and p38 MAPKs, (e) 4EBP-1, which is a substrate for the mTOR signaling pathway and (f) S6K1, which leads to mTOR activation (87). The activation of nAChRs can also occur through the recruitment of a scaffolding protein,  $\beta$ -arrestin-1.

Additionally, nAChRs might functionally network with  $\beta$ -AR and, consequently, the EGFR pathway (**Figure 3**). The indirect stimulation of  $\beta$ -AR via nAChRs has been reported in NNK-treated small airway epithelial cells (47).

### **Nitrosamine signaling implication in the metabolic reprogramming of cancer cells**

Alterations in cellular metabolism are considered an important hallmark of cancer, as this reprogramming can be observed in most types of cancer cells (88). In a simplified view, one could describe two main types of metabolic reprogramming: glycolytic and oxidative (89). Glycolytic tumors rely more on glycolysis to generate ATP as well as building blocks, with little contribution of the mitochondrion. Several mechanisms and determinants of the glycolytic phenotype have been described and reviewed in the last decade. Briefly, tissue hypoxia or oncogene mediated pseudo-hypoxia promotes the HIF1alpha-dependent expression of specific glycolytic genes and inhibits oxidative metabolism through PDK1 expression (90). In contrast, oxidative tumors rely on mitochondrial energy transduction from various sources of carbons as carbohydrates, amino-acids or fats. The determinants of the oxidative profile are also numerous and include specific oncogenes activation (as KRAS in lung tumors (91) or Myc in breast cancer (92)) as well as environmental factors as tissue perfusion (93) or oxidative stress (94). Cancer cells metabolic reprogramming can occur independently of metabolic substrates availability as exemplified by aerobic glycolysis or Warburg effect, where cancer cells use glycolysis even in presence of oxygen. The bioenergetic profile of a given tumor depend on several parameter and inter-patient as well as intra-tumor heterogeneity have been described (93). A large number of signaling pathways have been involved in the reprogramming of tumor energy metabolism but, surprisingly, little attention has been given to the role of nitrosamine signalling in this hallmark of cancer. As discussed above, several lines of evidence indicate that nitrosamines can activate the PI3K/Akt, Myc, HIF1alpha, KRAS and NRF2 signaling pathways through  $\beta$ -AR, nACHr and ROS-dependent or oncogenic-dependent signaling. These pathways also play a role in the control of energy metabolism, suggesting that nitrosamines could participate to the reprogramming of cancer cells bioenergetics, in concert with changes in other cancer hallmarks such as genomic and epigenomic alteration, deregulated cell proliferation and increased migration (**Figure 4**).

**PI3K/Akt** : The PI3K/Akt pathway, which is located downstream of receptor tyrosine kinase (RTK), is a crucial actor in metabolic reprogramming that leads to cell growth (161,163). Inappropriate activation of PI3K, which leads to activation of the pro-survival kinase Akt, is among the most common perturbations in various human cancers; several well-characterized oncogenes and tumor suppressors are located in this network (95). One of the consequence of activation of this pathway is the promotion of glucose uptake and glycolytic metabolism, directing cellular metabolism toward anabolic growth through Akt-mediated membrane translocation of glucose transporters, and Akt-dependent activation of hexokinase and phosphofructokinase (96). By phosphorylation and activation of ACL, Akt can stimulate the

conversion of citrate to acetyl-CoA and OAA, which leads to *de novo* fatty acid synthesis (97). A major consequence of the activation of this pathway is the activation of the cell growth regulator mTOR complex 1 (mTORC1). This downstream activation is mediated by the inhibitors of this regulator: TSC2 or proline-rich Akt substrate 40 kDa (PRAS40) (98). Activation of mTORC1 enhances protein synthesis by phosphorylation of the translational regulators 4E-binding protein 1 (4E-BP1) and S6 kinase 1 (S6K1) (98). Moreover, mTORC1 activation has an effect on the promotion of mitochondrial biogenesis and respiration, gluconeogenesis as well as many other metabolic processes. These effects can be modulated by a nuclear transcriptional coactivator, PGC1 $\alpha$  (99–102). mTORC1 also has an effect on a number of transcription factors that coordinate metabolic gene expression: HIF1 $\alpha$ , Myc, and sterol regulatory element-binding protein 1 (SREBP-1) (103).

The impact of NNK on the PI3K/Akt pathway will also depend on the micro-environment of the cell, in particular the availability to nutrients and to oxygen. As mentioned above, the PI3K/Akt pathway responds to external growth factors and hormonal signals by promoting glucose transport, aerobic glycolysis, and anabolic synthesis of macromolecules. Subsequently, in the presence of insufficient glucose to feed this pathway, the cell should find another source to supply its ATP demand. Adenosine monophosphate-activated protein kinase (AMPK) is a known sensor of cellular energy status that monitors ATP levels in the cell and is activated under energetic stress, when intracellular ATP decreases and intracellular AMP increases, such as during hypoxia or nutrient deprivation. Activation of this kinase stimulates a program of metabolic adaptation to maintain energy homeostasis (by limiting energy consumption or enhancing energy production) and thus allows adaptation to a given metabolic stress (104,105). In fact, upstream of this kinase is a serine-threonine kinase, liver kinase B1 (LKB1), which is a tumor suppressor and is considered an activator of AMPK. The LKB1/AMPK pathway, as a metabolic checkpoint in the cell, initiates suppression of the PI3K pathway by inhibiting the mTORC1 pathway and arresting cell growth under conditions of low intracellular ATP levels (106). mTORC1 inhibition is accomplished through phosphorylation of TSC2 and regulatory associated protein of mTOR (raptor)(107,108). By this inhibition, AMPK blocks protein translation and fatty acid synthesis, which consume ATP. Fatty acid synthesis can be blocked by acetyl-CoA carboxylase-1 (ACC1) phosphorylation (106). Another consequence of AMPK activation is the induction of autophagy (109). Moreover, fatty acid oxidation will increase to generate acetyl-coenzyme A (CoA) as a substrate for the TCA cycle and oxidative phosphorylation (96). In metabolic adaptation, some of the downstream effects of AMPK are related to phosphorylation and activation of p53 tumor suppressor. This tumor suppressor is one of the

most important transcription factors, playing a vital role in the response and regulating metabolic stress to prevent tumor development. p53 activation stimulates transcriptional activity and initiates a metabolic cell cycle checkpoint that restricts cell proliferation (110). As a vital cancer defender, p53 has a diverse range of functions in the promotion of oxidative phosphorylation and downregulation of glycolysis, and its mutation can lead to cancer development. Moreover, as p53 regulates cell growth and autophagy, its role is significant in the cellular response to starvation (111).

**HIF1 $\alpha$ :** the Hypoxia-induced factor 1-alpha (HIF1 $\alpha$ ) is responsible for metabolic adaptation in hypoxia. At first, during hypoxia the main reason to use glycolysis is the lack of oxygen and HIF-1 further increases glycolysis through the induction of glycolysis related genes (112). Enhancement of the glycolytic machinery involved the increased expression of glucose transporters, glycolytic enzymes, and of PDK1 that will in turn inhibit pyruvate dehydrogenase and glucose-dependent oxidative phosphorylation (90). The other transcription factor, Myc, which is activated downstream of mTORC1, also acts in the regulation of cell growth and proliferation (113). Myc targets genes that support the proliferative utilization of glutamine, including glutamine transporters, and those involved in both mitochondrial biogenesis and glutaminolysis. It can also stimulate enhanced expression of many genes involved in glucose uptake, glycolysis, and lactate production (114). Myc increases the expression of many genes that support anabolic growth, including transporters and enzymes involved in glycolysis, fatty acid synthesis, glutaminolysis, serine metabolism, and mitochondrial metabolism (115). Myc-transformed cells undergo apoptosis in the absence of exogenous glutamine (116–118). Moreover, Myc also induces the expression of enzymes in other anabolic pathways, such as serine hydroxymethyltransferase (SHMT) (serine/glycine metabolism) and fatty acid synthase (FAS)(lipid biosynthesis) (119). The last mentioned transcription factor, SREBP (sterol regulatory element-binding protein), plays a role by inducing the expression of several genes involved in fatty acid biosynthesis in response to growth factor signaling (120). As a downstream effector of mTORC1, SREBP1 can lead to the deregulation of *de novo* lipid synthesis (121).

**KRAS:** Regarding KRAS, NNK can induce activating-KRAS mutations and causes primarily lung adenomas (in susceptible mouse strains) (122). Ras is a type of small GTP-binding protein that functions as a component of several signaling cascades that function in other various signaling cascades, such as RAF/MEK/ERK, PI3K/Akt, and RalEGF/Ral pathways (123). RAS has different subtypes, such as H-RAS, N-RAS and K-RAS, and the effect of each of them differs in mediating different signaling pathways. These genes are commonly mutated in human cancer, leading to cellular proliferation (32,124). KRAS

mutation is more frequent in comparison to the other subtypes in most cancer types (including lung adenocarcinoma) (125). Activation of KRAS can increase the glycolytic flux but decreases glucose consumption in the TCA cycle and associated anabolic pathways. Instead, to sustain biosynthetic reactions, this oncogene activation promotes the utilization of glutamine for anabolic pathways (126). Thus, in KRAS mutated cells, glutamine can be used as both carbon and nitrogen sources for biosynthetic reactions to sustain cell growth and quench ROS production metabolism, leading to an efficient utilization of both carbon and nitrogen and in this way playing an essential role in the proliferation and survival of cancer (127–129). Through MEK/ERK kinases activation, KRAS can phosphorylate proteins directly or promote protein phosphorylation and consequently regulate various cellular functions (130). ERK signaling downstream of KRAS mutation can lead to lipogenesis and alter lipid metabolism in cancer (131). The proposed target in this pathway inducing lipogenesis is fatty acid synthase (FASN) (126). Additionally, the PI3K/Akt/mTOR pathway is critical for NNK-induced lung tumorigenesis (132).

**NRF2:** The potential role of **NFE2-related factor 2 (or nuclear respiratory factor) (NRF2)** in NNK-mediated metabolic reprogramming was suggested by studies showing that NNK can induce oxidative stress in lung tissues in mice and rats by creating a major premutagenic lesion, 8-hydroxy-2'-deoxyguanosine (8-OHdG), which is considered a marker of DNA oxidative damage produced by ROS (133–136). ROS are intracellular chemical species that contain oxygen and include the superoxide anion ( $O_2^-$ ), hydrogen peroxide ( $H_2O_2$ ), and hydroxyl radical ( $OH\cdot$ ). As these radicals have a single unpaired electron, they are highly reactive (137). The initiator of ROS in aerobic cells is  $O_2^-$ , which is generated from the incomplete reduction of molecular  $O_2$  to  $H_2O$  in the mitochondrial respiratory chain and peroxisomal oxidation (138) (and cytosolic NADPH oxidases (NOXs) (139)). After  $O_2^-$  generation, it can be converted to  $H_2O_2$ , which is facilitated in this reaction by superoxide dismutase 1 or 2.  $H_2O_2$  can be converted either to  $H_2O$  (catalyzed by peroxiredoxins (PRXs) and consequently detoxified, or to  $OH\cdot$  in the presence of ferrous or cuprous ions.  $OH\cdot$  can lead to the oxidation of lipids, proteins, and DNA, which consequently damage cells (140). Low amounts of ROS can be considered a positive regulator of cell proliferation and cellular adaptation to metabolic stress (particularly  $H_2O_2$ ) (141), while higher amounts of ROS can lead to cell death signaling pathways, initiated by  $H_2O_2$  and followed by  $OH\cdot$  generation, which can damage cellular components. In such conditions of oxidative stress the main function of NRF2 is to activate the cellular antioxidant response. Thus, NRF2 is an important regulator of cell survival and is considered a main defense system of the cells (142). The primary regulator of NRF2 is Kelch-like ECH-associated protein 1 (KEAP1). KEAP1 is a



substrate adaptor for a Cul3-containing E3 ubiquitin ligase. KEAP1 and the E3 ubiquitin ligase complex degrade NRF2 to maintain its low level. High levels of fumarate can also cause KEAP1 inactivation and consequently stabilize NRF2. Excess NRF2 binds to antioxidant response elements (AREs) and turns on nDNA stress-response genes, including haem oxygenase 1 (*HMOX1*), which appears to be important in tumorigenesis. NNK can stimulate the generation of mitochondrial ROS and mimic the effects of hypoxia to induce HIF1 $\alpha$  accumulation and activity in lung cancer cell lines (143). In cancer cells, DNA lesions can also lead to excess generation of ROS. Higher ROS levels can stimulate the PI3K and MAPK/ERK signaling pathway and activate transcription factors such as HIF1 $\alpha$  and NF- $\kappa$ B (144–146). Many lines of evidence indicate that levels of intracellular ROS were closely linked to HIF1 $\alpha$  stabilization, possibly through the modification of prolyl-hydroxylase (PHD) activity (147). Furthermore, mitochondria, which are the main source of ROS production, appear to play a critical role in the regulation of HIF1 $\alpha$  stability (148). Intratumoral hypoxia can cause metastasis, which leads to cancer progression (149). Based on the available evidence, most cellular consequences related to hypoxia are mediated by the HIF1, which consists of two proteins — HIF1 $\alpha$  and HIF1 $\beta$ . These two proteins can bind to hypoxia response elements (HREs) in the promoter region of numerous metastasis-associated genes and in this way promote metastasis (147,150). HIF1 $\alpha$  degradation under normoxia is regulated by PHD enzymes, growth factor stimulation and free radical generation.

***$\beta$ -AR- and nAChR-dependent regulation of mitochondrial biology:*** Biochemical studies have shown that mutations in  $\beta$ -AR receptor can increase cell respiration and fatty acid oxidation (151). This increase in cell respiration as a result of activation of  $\beta$ -AR associated with an increased production of ROS (152). It is also demonstrated that  $\beta$ -AR signaling through agonist binding can induce mitochondrial biogenesis (153–156) and in this way modulate oxidative metabolism, energy expenditure, lipolysis, **glucose transport, and glucose oxidation** (157–159). The regulation of mitochondrial biogenesis by nitrosamine signaling could involve stimulation of the nuclear transcriptional coactivator peroxisome proliferator-activated receptor  $\gamma$  coactivator 1- $\alpha$  (PGC-1 $\alpha$ ). This transcriptional coactivator interacts with CREB and nuclear respiratory factors 1 and 2 (NRF1 and 2) (160). As discussed above, CREB is activated by stimulation of Beta-adrenergic signaling (**Figure 2**). The transcription factor CREB can induce the expression of a series of genes involved in mitochondrial biogenesis and mitochondrial respiration (161–163). CREB participates to the so-called retrograde signaling involved in the stimulation of mitochondrial function in response to increased energy needs (163). Studies in neurons further showed that CREB can act directly on mitochondrial DNA-encoded gene transcription (164), raising the need to

investigate this possibility in the context of cancer. Likewise, the SRC kinase and STAT3, two components of the non-canonical activation of  $\beta$ -AR signaling have also been related to the stimulation of mitochondrial function. For instance, SRC kinase has been discovered inside the mitochondrion where it could phosphorylate a complex-I subunit, leading to changes in cell respiration (165,166). Similar findings have been reported for STAT3 with a mitochondrial-form of this transcription factor involved in the stimulation of OXPHOS (67,167). It is also suggested that  $\beta$ -AR can regulate mitochondrial function directly and indirectly through cAMP production. In this context, cAMP activates various effectors like PKA and Epac that lead to PGC1 $\alpha$  activation (168–173). Moreover, other B-AR signaling could trigger mitochondrial biogenesis through Ca<sup>2+</sup> and CamKK $\beta$  or AMPK (174–177).

Regarding nAChR, cell biology studies have revealed that this receptor is expressed on mitochondrial outer membrane of non-neuronal cells (178). It was also demonstrated that mt-nAChR is coupled with voltage-dependent anion channel (VDAC) and regulate Ca<sup>2+</sup> accumulation and cytochrome *c* release during apoptosis. The expression of mt-nAChR can also regulate mitochondrial permeability transition (179) and it was proposed that activation of mt-nAChR by some agonists that can permeate the cells (such as nicotine) may inhibit mPTP opening to reduce apoptosis (178–181).

## Conclusion

Cigarette smoke, which is the predominant form of tobacco consumption, is the largest preventable cause of cancer, attributed to approximately 30% of all cancer deaths (182–184). Cigarette smoke has a complex composition, and the exact mechanism by which these components can lead to carcinogenesis is still not well understood. To understand the molecular events related to cigarette smoke, several epigenetic (185–187), genomic (188–190), transcriptomic (191,192), proteomic (193–195) and metabolomic (196,197) studies have been conducted. **In particular, metabolomics showed that tobacco smoke immediately (2 hours) leads to altered level of glycerophospholipids, glutamate and 2-octenoylcarnitine in the blood, suggesting changes in lipid and glutamine metabolism (190). Conversely, long-term effect studies identified specific metabolites that varied in proportion with tobacco-smoke exposure (189). These included nicotine metabolites and xenobiotic metabolites involved in benzoic or xanthine metabolism as well as specific amino acids, lipids, vitamins or cofactors and one carbohydrate. No metabolite from glycolysis or TCA cycle were identified in these studies. However, the specific changes that occur in lung tissue were not investigated in these studies focused on the systemic changes induced by tobacco-smoke. Therefore, metabolomics**

studies will be required on lung tissue of animal model expose to TSNAs to evidence potential changes at the level of energy metabolism.

Cancer metabolism, an emerging hallmarks of cancer, is an important aspect of cancer initiation and development, the reprogramming of which is often a consequence of tumorigenic mutations and epigenetic alterations (198). Further dissecting the role of cancer metabolism and tracking its plasticity in addition to other molecular aspects of cancer studies can help us to better understand the pathobiology of cancer, which will in turn lead to the identification of early detection biomarkers and/or therapeutic targets. Cigarette smoke has been reported to enhance lactate production in human bronchial epithelial cells and lead to glucose consumption (199). Moreover, one of the most important effects of cigarette smoke is the induction of oxidative stress. Activation of aryl hydrocarbon receptor (AHR) by cigarette smoke can be associated with the expression of oxidative stress and antioxidant genes (200–203), as documented in an human airway epithelium cell line (*i.e.*, activation caused increased ROS production (204)). AHR is a cytosolic ligand-activated transcription factor that is activated upon encountering multiple foreign ligands, such as cigarette smoke. Activation of AHR can lead to the transcription of genes related to biotransformation enzymes such as CYP1A1. A prominent role of CYP1A1 is related the metabolism of some carcinogens found in cigarette smoke, such as nitrosamines and poly aromatic hydrocarbons (205). Upon chronic exposure of lung cancer cells to cigarette smoke, higher expression of AHR regulatory genes are observed, followed by an alteration in mitochondrial proteins, specifically enzymes involved in TCA and OXPHOS. Moreover, this chronicity leads to higher expression of glutamine synthase, fatty acid degradation and lactate synthesis (195), in addition to reduced expression of many glycolytic enzymes such PKM2 (195), which is a rate-limiting glycolytic enzyme and acts as a key component in tumor metabolism and growth. As a consequence of the lower activity of this enzyme, the upstream intermediates of glycolysis will accumulate and enter anabolic pathways (206). As higher ROS production can lead to inhabitation of PKM2 (207), mitochondria exposed to cigarette smoke are metabolically altered to support their survival and proliferation (195). In general, there is a body of evidence that indicates cigarette smoke plays a potential role in mitochondrial metabolic reprogramming (195). All these reports indicate that cigarette smoke plays a profound role in metabolic alterations. Several studies have also examined the role of each carcinogenic component of tobacco smoke separately, such as the investigations of TSNAS reviewed in this article. Despite several reports on this subject, little is known about their mechanisms, especially regarding metabolic reprogramming. This suggests that more research in the field is necessary.

## Figure Legends.

**Figure 1. Schematic illustration of the NNK and NNN pathways producing DNA adducts.** Via P450s, NNK is metabolized to  $\alpha$ -hydroxy NNK or can undergo reduction to NNAL, which is converted to NNAL glucuronides (NNAL-Glucs). Like NNK, NNAL is metabolized to  $\alpha$ -hydroxy NNALs by P450s, and then both  $\alpha$ -hydroxy NNKs and  $\alpha$ -hydroxy NNALs are decomposed to diazonium ions and aldehydes. NNN undergoes  $\alpha$ -hydroxylation, as well by P450s and is converted to  $\alpha$ -hydroxy NNNs.  $\alpha$ -hydroxy NNKs,  $\alpha$ -hydroxy NNALs and  $\alpha$ -hydroxy NNNs are unstable products, and they are rearranged into diazonium ions, which are highly reactive and produce DNA adducts. If DNA adducts are not repaired they can cause miscoding followed by mutational activation of oncogenes and/or inactivation of tumor suppressor genes. As a consequence, these mutations lead to loss of normal cellular growth control functions which results in cellular proliferation and cancer (208). DNA adducts formation happen both in nuclear DNA (nDNA) and mitochondrial DNA (mtDNA) (209). Based on critical role of mitochondria in cellular energy production, apoptosis, and cellular growth and differentiation, its alteration may be involved in the carcinogenic process. These alterations can happen due to accumulation of some carcinogens in mitochondria that can bind to mtDNA and remain because of low-efficiency mtDNA repair mechanisms (210,211). There is growing body of evidence that confirm the role of mtDNA mutations in cancer. Such evidence includes the link between mtDNA mutations and constitutive oxidative stress in cancer cells. Moreover, in malignant cells, mtDNA abnormalities and alterations in respiratory activity seems a general feature. These mutations can changes the cell surface produced by mtDNA mutations in cancer cells. Abnormal expression of mtDNA-encoded proteins due to mutations and deletions in mtDNA, have been observed in various solid tumors (212). It is reported that pyridyloxobutyl (POB)(213) and pyridylhydroxybutyl (PHB) (214) DNA adducts form and accumulate in NNK chronically treated rats (209). Based on this study, NNK treatment in rats gives higher levels of POB and PHB adducts in mtDNA than in nDNA of their lungs (209). NNK can form POB-DNA adducts via methyl hydroxylation. It can also convert to NNAL by carbonyl reduction. NNAL can form PHB-DNA adducts via hydroxylation of the methyl group (215,216). Results from this study support the hypothesis that tobacco carcinogens can bind to mtDNA and consequently develop smoking-induced lung cancer (209).

**Figure 2.  $\beta$ -adrenergic receptor (B-AR)-mediated nitrosamines signaling.** Binding of TSNA as agonists to  $\beta$ -AR on cancer cells activates GTP-binding proteins (G-proteins) coupled to receptor signaling, resulting in the activation of adenylyl cyclase and cyclic AMP

(cAMP) followed by protein kinase A (PKA) activation. In turn, PKA triggers the MAPK cascade and AA release. Conversely, cAMP induces the release of epidermal growth factor (EGF) and transactivation of EGFR signaling. Downstream of EGFR, PI3K/Akt pathways may be activated. All these pathways can activate downstream effectors that lead to the promotion of cell proliferation and inhibition of apoptosis and migration.

**Figure 3. Nicotine/Acetylcholine receptor (nAChR)-mediated nitrosamine signaling.**

Binding of TSNA to nAChRs causes a change in the conformation of the receptor subunits, which opens the receptor gates. Opening the gates,  $\text{Ca}^{2+}$  flows into the cell, which leads to membrane depolarization. This depolarization is enhanced by activation of the gate of voltage-activated  $\text{Ca}^{2+}$ -channels and greater influx of  $\text{Ca}^{2+}$ . The increased intracellular  $\text{Ca}^{2+}$  concentration triggers EGFR/RAF/MAPK, PI3K/Akt signaling pathways. Moreover, this depolarization leads to the release of the autocrine growth factor serotonin and the angiogenic factor VEGF. Serotonin can also activate PKC and its downstream RAF and the MAPK cascade. All these pathways can induce activation of different transcription factors and, consequently, regulate gene and protein expression; this leads to the stimulation of cell proliferation and migration and inhibition of apoptosis. In addition, nAChR can indirectly stimulates  $\beta$ -AR signaling through the release of adrenaline and noradrenaline.

**Figure 4: Regulation of energy metabolism by tobacco-smoke derived nitrosamines (TSNAs).**

Two main mechanisms can relate nitrosamine signaling to the regulation of energy metabolism. On the one hand, TSNAs activate the beta-adrenergic and the acetylcholine-nicotine receptors to activate a series of transcriptional regulators (pink box) that will in turn trigger the expression of various energy genes involved in glucose metabolism, lipid metabolism, autophagy and mitochondrial function. On the other hand, TSNAs induce oxidative stress and genotoxicity as well as oncogenic activation that also induce a metabolic response. Fine tuning of the metabolic response to TSNAs exposure will depend on the cellular context, the microenvironment and the type and dose of exposure.

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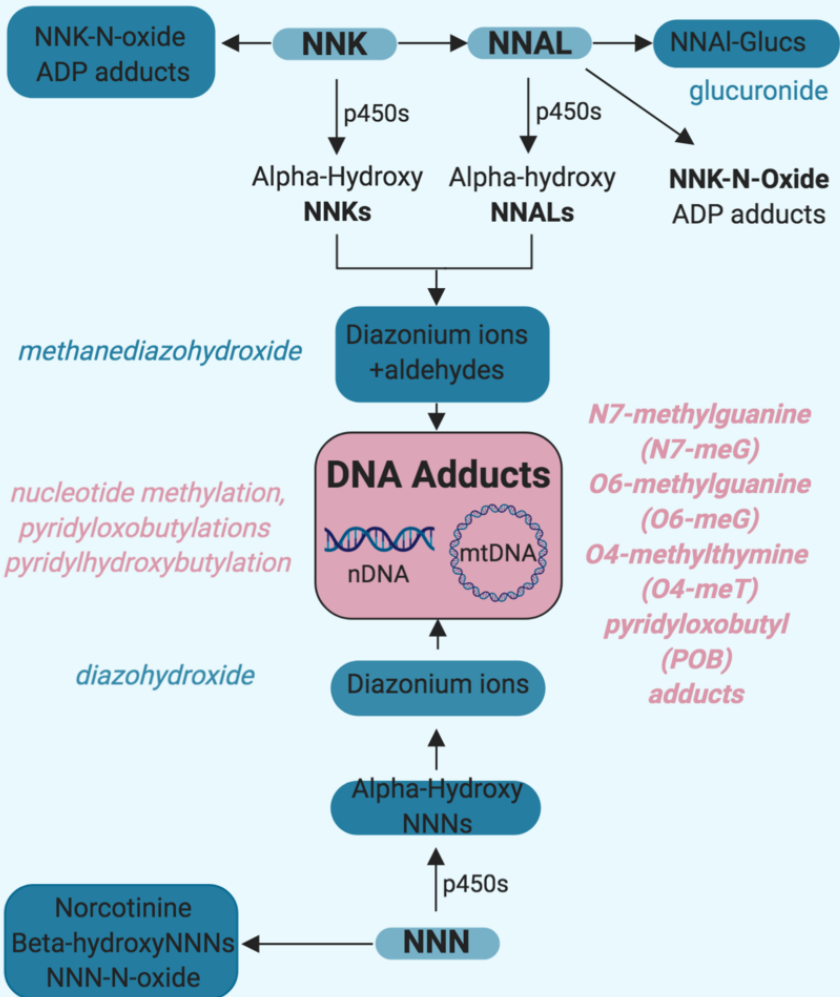


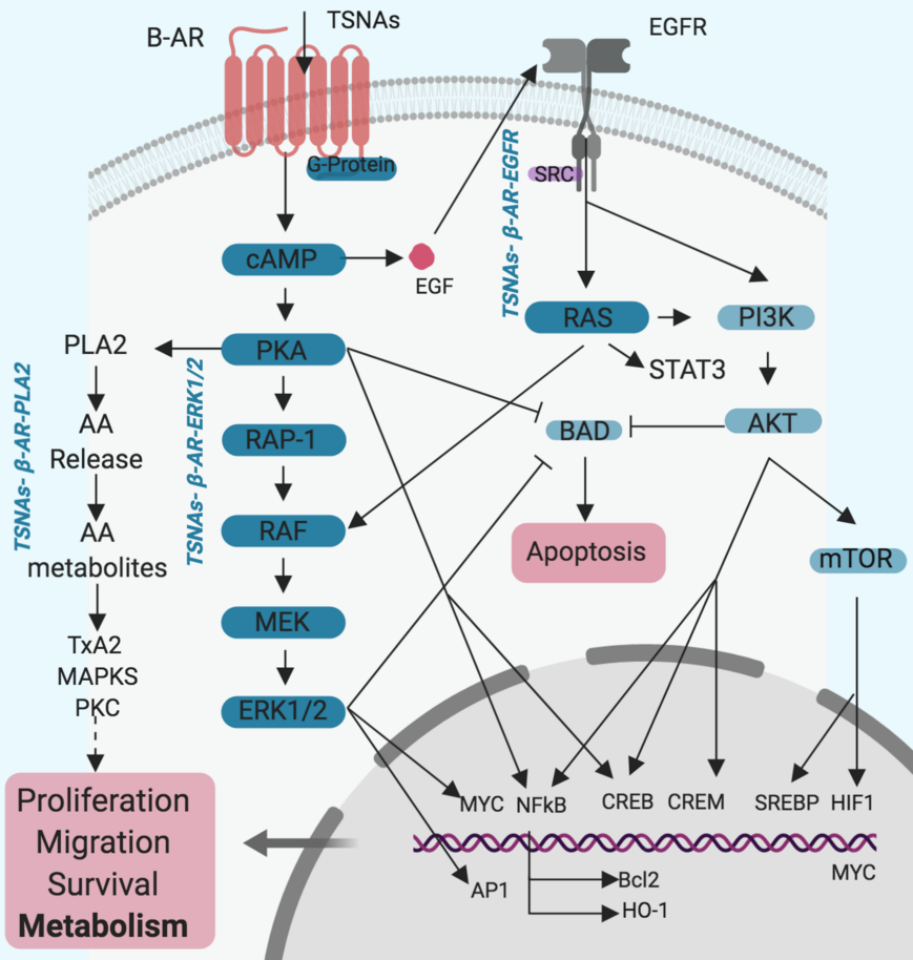
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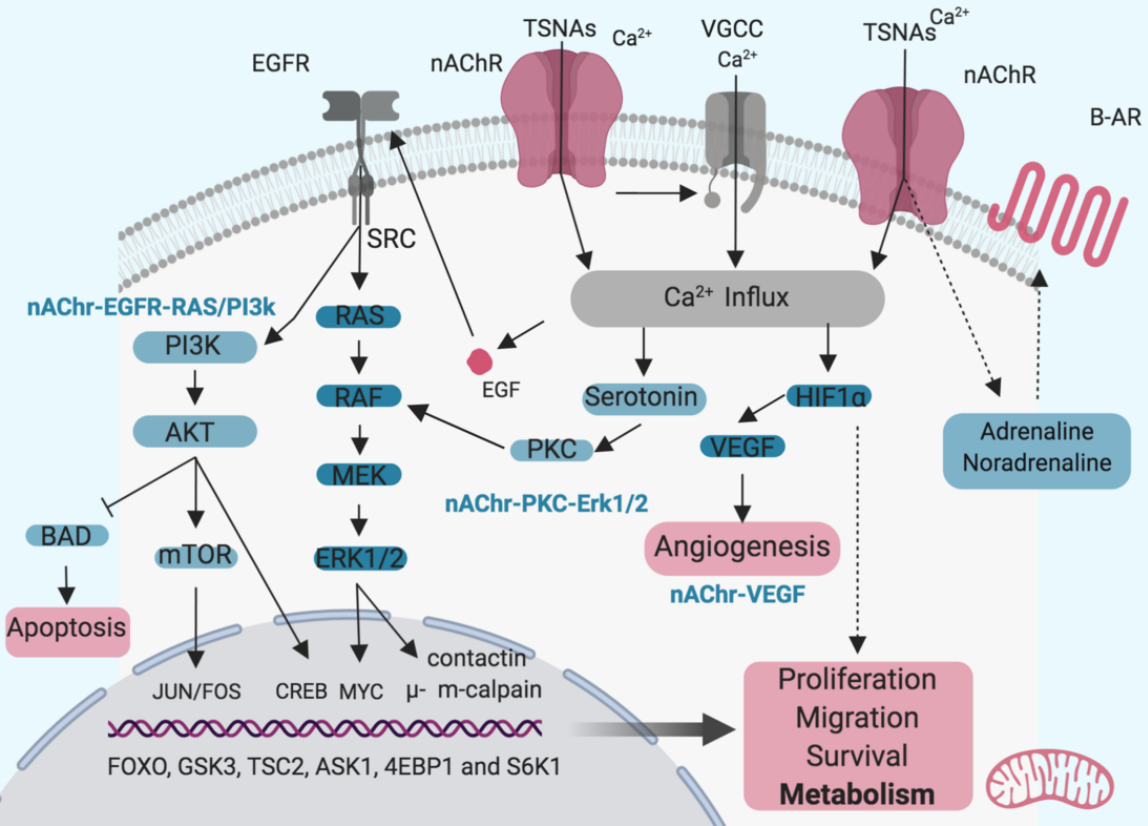
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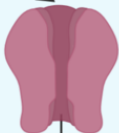




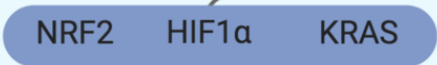
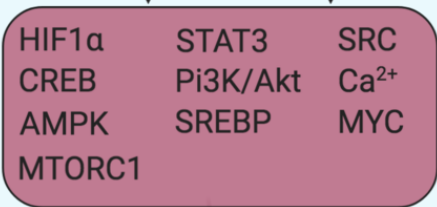


# TSNAs

nAChR



B-AR



*Oxidative stress*

*Chromosomal instability*

**TSNAs**