

## MINI REVIEW

# Expression and role of p16 and GLUT1 in malignant diseases and lung cancer: A review

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## Introduction

Lung cancer is the leading cause of cancer-related death.<sup>1</sup> Adenocarcinoma is the most common histotype, followed by squamous cell carcinoma and large cell carcinoma.<sup>2</sup> The broadest classification of lung cancer is in small and non-small cell cancer (NSCLC) which is the prevalent subtype.

Cigarette smoking is by far the main avoidable cause of both NSCLC and small cell lung cancer (SCLC) often preceded by lung nodule detection.<sup>3–5</sup> It is estimated that the prevalence of NSCLC will continue to rise, partially due to the rise in tobacco use and also to an increase in air pollution.<sup>6</sup>

NSCLC is usually symptomatic and is therefore diagnosed at an advanced stage. Despite recent advancement in therapies, the prognosis of NSCLC patients remains unsatisfactory with a five- or 10-year survival rate of 15%–20% of the total population without *EGFR* gene mutation or *ALK* translocation.<sup>7</sup>

Over the last decades many screening programs and new techniques for early detection have been developed to improve the prognosis.<sup>8</sup> Some criteria have been identified

## Abstract

Non-small cell lung cancer (NSCLC) is the leading cause of cancer death and in most cases it is often diagnosed at an advanced stage. Many genetic and microenvironmental factors are able to modify the cell cycle inducing carcinogenesis and tumor growth. Among the metabolic and genetic factors that come into play in carcinogenesis and tumor cell differentiation and growth there are two different proteins that should be considered which are glucose transporters (GLUTs) and p16<sup>INK4</sup>. The first are glucose transporters which are strongly involved in tumor metabolism, notably accelerating cancer cell metabolism both in aerobic and anaerobic conditions. There are different subtypes of GLUT family factors of which GLUT 1 is the most important and widely expressed. By contrast, p16 is mainly a tumor-suppressor protein that acts on cyclin-dependent kinase favoring cell cycle arrest in the G1 phase. Our search focused on the action of the aforementioned factors.

as being associated with a high risk of patients developing lung cancer such as a smoking habit of a pack-year of more than 20, family history of cancer, characteristics and location or shape of lung nodules.<sup>9</sup>

New prognostic and target molecules need to be determined to predict lung cancer development and evolution.

Our study focused on two different proteins and their associated genes both involved in a different manner but at the same time resulting in lung carcinogenesis and tumor growth.

Glucose transporters (GLUTs) are a family of proteins that favor the transport of glucose in the blood. Several members of glucose transporters have been identified from GLUT-1 to GLUT-12.<sup>10</sup>

GLUT proteins have both an intracellular and transmembrane domain. Several conditions and factors are able to stimulate the aforementioned, such as hypoxia and inflammatory factors such as IL-6, and VEGF.<sup>10</sup>

p16 protein is an antioncogene, recognized as a tumor suppressor whose action takes place by inhibiting the phosphorylation of the retinoblastoma protein (pRb) by

binding to the cyclin-dependent kinase complexes (CDK4–CDK6) which leads to arrest in phase G1 of the cell cycle (Fig 1).<sup>11</sup>

## Methods

A literature search was carried out using the main databases such as Pubmed, Google Scholar, and Scopus with lung cancer pathways, GLUTs and p16 as keywords. We also included some criteria found in English language papers published by peer reviewed journals, as well as studies carried out in humans, in vitro and in vivo focusing on the expression of GLUT and p16 in metastatic malignant cancers. Clinical trials or cohort studies were included if the considered endpoint was survival with a sample size of at least 50 patients. Studies concerning patients with severe comorbidities were excluded.

The differences in GLUT and p16 expression are reported in this review.

## Results

### p16

Among the proteins and factors involved in carcinogenesis and tumor cell proliferation the role of p16 should be taken into account.<sup>11</sup> The locus where p16 gene is included is located on human chromosome 9p21 and is frequently subjected to deletions hindering its action as a tumor suppressor. Its main function is in cell cycle regulation inhibiting the pRb pathway by downregulation of cyclin-dependent kinases.

### p16 in human studies

p16 protein and its related gene are associated with HPV infection and its activity has been studied in different cancers including cervical cancer.<sup>12</sup> The p16 positive expression associated with HPV infection has been studied as a predictive factor in different cancers such as head and neck cancer, showing its association with a better response after radiotherapy.<sup>13</sup> Another type of cancer in which p16 plays a role is oropharyngeal cancer; indeed, it has been found to be a prognostic factor associated with a better outcome and a decreased epidermal growth factor (EGFR) expression.<sup>14</sup> In vitro studies have confirmed that there is a relationship between p16 and EGFR activation.<sup>15</sup> Expression of p16 protein has also been found to affect patient survival in tonsillar carcinoma.<sup>16,17</sup>

Investigating its action, p16 inhibitor kinase 4 (p16-INK4a) protein has a negative regulatory role in the cycle of eukaryote cells by inducing their arrest during the course of differentiating processes.<sup>18</sup> Conversely, p16 gene inhibition leads to phosphorylation of pRb, unblocking the cell cycle with a

subsequent uncontrolled cell growth and increased proliferation in all cancer types.<sup>19</sup> Several genetic alterations can induce inactivation of p16 including homozygous deletions, promoter hypermethylation, point mutations and loss of heterozygosity (LOH).<sup>20</sup> Homozygous deletions and promoter hypermethylation lead to its dysregulation, whereas point mutations and small deletions, in particular missense mutation, alter the structure and activity of p16.<sup>21</sup> Thus, p16 deregulation is favored by different transcriptional factors and oncogenes. Genetic inactivation of p16 may be compensated by the activation of other antioncogenes such as p21. This suggests that carcinogenesis is the result of an imbalance between oncogenes and oncoprotein activation and their inhibition.

There are different subtypes of inhibitors of cyclin-dependent kinases such as p16INK4a, p15INK4b, p18INK4c, all of which are able to affect the cell cycle.<sup>22</sup> There is a cross-talk among p16INK and transcription factors such as NF-κB which induces cell growth and cancer proliferation. p16 is able to compete with NF-κB to bond with cyclin-kinases.

Previous studies have demonstrated that p16-INK4a is overexpressed in cancers and acts as a tumor suppressor. It is closely associated with cell death and tumor aggressiveness.<sup>23</sup> The downregulation of p16 leads to cancer progression but overexpression is associated with poor prognosis in different solid cancers.

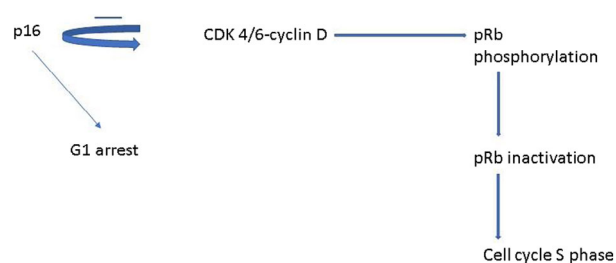
### Preclinical studies

The p16 gene is CDKN2A which encodes two different genes - p16 INK4a gene and p14ARF - which in turn is involved in apoptosis.<sup>24</sup> Their common function is to inhibit cell cycle progression. Some amino acid residues of p16 have been identified that interact with cyclin D-dependent kinases cdk4 and cdk6 inhibiting pRb phosphorylation.<sup>25</sup>

An antiapoptotic role played by p16 has been shown to be associated with downregulation of proapoptotic proteins such as Bax-Bak and cell cycle regulator Bcl-2, and sensitization of proliferating cancer cells to cisplatin activity.<sup>26</sup> The latter is an antineoplastic agent which acts by alkylating cancer cell DNA, and its action is sensitive to different agents. The involvement of Bax in the apoptotic process counterbalances the action of procarcinogenic molecules. In Table 1 the main factors activated with the associated effects are represented and Fig 1 depicts the main pathway.

### p16 in lung cancer

p16 tumor suppressor undergoes mutational and epigenetic alterations in several human cancers and acts as a critical target for the inactivation of several DNA tumor viruses.



**Figure 1** p16 is able to inhibit the action of cyclin D kinase which is responsible for retinoblastoma protein (pRb) phosphorylation leading to pRb inactivation and cell cycle progression stimulating the S phase. By contrast, p16 action leads to G1 arrest of the cell cycle.

**Table 1** Effects of p16

Factors involved	Pathway
pRb hypophosphorylation	Inhibition CDK4 and CDK6 and G0 arrest
P21 activation	Cell cycle arrest
P53 coactivation	G2 cell cycle arrest
P15-INK4B coactivation	Tumor-suppressor

### Preclinical studies

A potential role for p16 has been found in early lung cancer detection and preneoplastic lesion evaluation. It has been found to be useful in the discrimination between adenoma and adenocarcinoma when associated with heterogeneous nuclear ribonucleoprotein such as hnRNP, cyclin D1 and ki67.<sup>26</sup> Overexpression of cyclin D1 and decrease of p16 have been reported to be frequently observed in both adenocarcinoma and adenomatous hyperplasia.<sup>26,27</sup>

The interaction of p16/pRb as oncosuppressor genes has been found to be correlated with telomerase activity in adenocarcinoma of the lung in adenocarcinoma cell lines.<sup>28</sup> The relationship between p16 and activating mutation markers could influence the stepwise progression of tumor cells, inhibiting the mammalian target of rapamycin (mTOR) pathway and downstream activation of hypoxia factor.<sup>29</sup>

p16 as a tumor suppressor gene has been shown to interact with P53 increasing the action of cell cycle regulation.<sup>30</sup> The observation that p16 gene preferentially undergoes gene silencing through promoter hypermethylation is of crucial importance in the evidence of protein expression in NSCLC.<sup>31</sup>

### Studies in humans

The protein expression level in surgically-treated NSCLC has been reported to be associated with a poor prognosis probably due to a compensation to its inactivation by tumor factors.<sup>32</sup>

p16-INK as well as p40 and p63 has been found to be overexpressed not only in malignant but also in benign lung adenoma.<sup>33</sup>

Among the malignant lung diseases, abnormal expression of p16 has been found mainly in NSCLC in which methylation of p16 gene is frequently observed.<sup>34–36</sup> Hypermethylation of the p16 gene together with p53 and KRAS mutation has been reported to promote lung carcinogenesis in smokers.<sup>37–39</sup>

The p16 protein has been associated with the X-linked inhibitor of apoptosis protein (XIAP) in both benign and malignant neoplasm such as adenoma, and bronchiolo-alveolar carcinoma of the lung.<sup>40,41</sup> The X-linked inhibitor of apoptosis has, in turn, a prognostic role in NSCLC and has a relationship with antiapoptotic molecules such as caspase. The overexpression of XIAP is able to revert the action of caspase. Thus there is a network of genetic and transcriptional factors that cross-talk and influence cell growth and death.

## GLUT protein

### Preclinical studies

Among the GLUT isoforms there is a significant elevation in the level of GLUT-3 and GLUT-5 mRNAs in tumor cells, notably in hypoxic conditions, meaning that cancer cells increase their glucose intake and have higher metabolism rates. These isoforms are differently but ubiquitously distributed in several tissues. The rate of glucose transport depends upon changes in glucose gradient as demonstrated in in vitro and in vivo experiments.<sup>42,43</sup>

Elevated GLUT1 expression has been previously described in many cancers, including hepatic, pancreatic, breast, esophageal, brain, renal, lung, cutaneous, colorectal, endometrial, ovarian, and cervical.

### In human and in vitro studies

The isoforms GLUT 1 and 3 have been reported to be associated with poor survival.<sup>42,43</sup>

Moreover it has recently been shown that GLUT-3 as well as GLUT-1 are hypoxia-responsive in neural stem cells.<sup>43,44</sup>

The expression of GLUT-5 has been reported to be upregulated during hypoxia exposure to 1% O<sub>2</sub> tension leading to a 3.3-fold increase in the uptake of 2-deoxyglucose by adipocytes.

Increased glucose transport in malignant cells has also been associated with increased expression of glucose transporters, with overexpression of GLUT1 and/or GLUT3.<sup>45</sup>

The GLUT family is divided into three classes based on their sequence homology. They can be stimulated by

different molecules and their expression is located both intracellularly and at the cell surface.

Class I comprises the well characterized GLUT-1–GLUT-4. Class II comprises the fructose transporter GLUT5 and three recently described proteins, GLUT7, GLUT9, and GLUT1 which have been implicated in metabolic disorders.<sup>46</sup>

Class III comprises newly described proteins, GLUT-6, GLUT-8, GLUT-10, GLUT-12.

Each of the glucose transporter proteins display different affinities for glucose and other hexoses such as fructose, galactose, mannose, glucosamine. GLUT1 gene is responsible for basal glucose uptake and is expressed in virtually all tissues under normal conditions<sup>47</sup> GLUT2 is encoded by the SLC2A2 gene, with a low affinity for glucose and high-affinity for glucosamine.<sup>48,49</sup>

It has been shown in *Xenopus* oocytes that GLUT1, GLUT3 have a low affinity transport capacity for galactose, mannose and fructose and a high affinity for glucose. Tumor cells prefer to convert glucose into lactate instead of utilizing the mitochondrial metabolism and the oxidative phosphorylation chain for energy production.<sup>50,51</sup>

## In human studies

Earlier studies have demonstrated the presence of mRNA from different GLUTs in human tumors and a significant increase in the mRNA for GLUT1 in gastrointestinal cancers associated with an increased cell glycolytic metabolism.<sup>52</sup>

GLUT-1 is the only isoform which links with tumor grade and standard uptake value (SUV) index on FDG-PET test.<sup>53</sup>

The overexpression of GLUT-1 has been found to be associated with fluorodeoxyglucose (FDG) uptake, notably in pancreatic carcinoma.<sup>54</sup> Studies carried out on culture cells showed enhanced GLUT-1 expression along with glycolytic enzymes in the presence of oncogenes. The other GLUT isoforms including GLUT-2, GLUT-3, GLUT-4 did not change their expression comparing healthy tissue with tumor. An activation and at the same time a decreased degradation were found suggesting a potential prognostic and predictive role.

Another cancer in which GLUT-1 expression was found to increase concurrently with high FDG uptake was breast cancer.<sup>55</sup> GLUT-1 was expressed on the cell membrane and cytoplasm, but unlike GLUT-2, is able to discriminate between benign and malignant disease.

Among various types of tumor, neck and head tumor often express both isoforms GLUT-1 and GLUT-3.<sup>56</sup>

Mitogens, oncogenes, and growth factors are all able to stimulate glucose metabolism and glucose transporters especially in tumors characterized by a high cell growth rate.<sup>57,58</sup> The glucose transport rate influences tumor growth by acting on cell differentiation, transformation and mitosis. Different tissues such as liver, brain,

**Table 2** Effects of GLUT-1

Factors activated	Effects
HIF 1- $\alpha$	Tumor progression, neoangiogenesis cancer metabolism
AMPK phosphorylation	Altered tumor cell metabolism
Cyclin E2	Cell cycle progression G0-G1
mTOR	Proteins synthesis, cell growth
KRAS mutation	Carcinogenesis, chemoresistance
PI3K	Increased glucose uptake and cancer metabolism
cMYC	Activation of glycolytic enzymes
EGFR expression	Tumor progression
FAK phosphorylation	Cancer cell migration

adipocytes, and small bowel present high mRNA expression of different isoforms.<sup>59</sup>

The induction of glucose transporters is tightly linked with the induction of hypoxia-inducible factor 1-alpha (HIF-1 $\alpha$ ), a factor able, in turn, to increase tumor metabolism, stimulate vascular angiogenesis and mitosis associated factors.<sup>60</sup>

A GLUT-1 associated enzyme, the phosphofructo-2-kinase contains binding sites for HIF in hypoxia condition, thus confirming the tight relationship between these two conditions of high glycolytic metabolism and hypoxia.<sup>61</sup>

Furthermore, glucose deprivation favors the development of KRAS mutations in cancer which is an oncogene often associated with aggressive nonresponsive tumors.<sup>62</sup> A condition of wild-type for KRAS gene is associated with low-glucose intake leading to reduced cell survival. KRAS mutation occurs in several malignancies such as colorectal, pancreatic and lung cancer. Clones with mutant KRAS present with an upregulation of GLUT-1 gene and are associated with a poor patient prognosis. Table 2 represents the main factors activated by GLUT-1 with its relative effects and Fig 2 shows the main pathways.

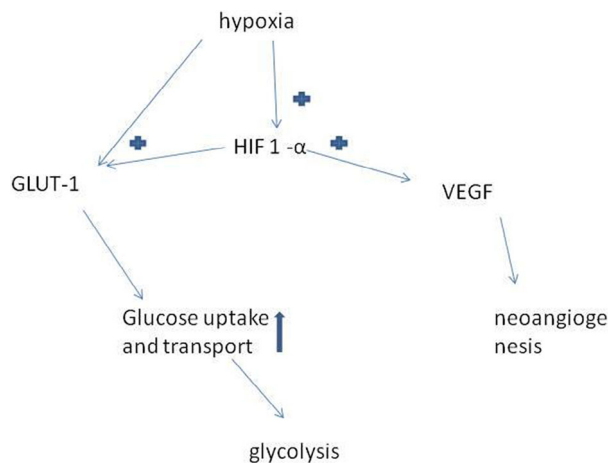
## GLUT 1 in lung cancer

Lung cancer is the leading cause of cancer death and different markers involved in cancer activation pathways are recognized to have a prognostic significance.<sup>63</sup>

## Preclinical studies

Indeed, transcription factors, oncogenes, cell cycle related genes participate in the process of tumor initiation and invasiveness, such as p21, Bcl-2, p53 and cyclin D.

Adding GLUT-1 RNA to lung cancer cell line A549 leads to a decrease of the expression of cyclins CDK 2 and 4 and conversely to an increased expression of p53 and 130 meaning that GLUT could affect cell cycle regulation.<sup>64</sup>



**Figure 2** Effects of GLUT-1 in relation to hypoxia. Hypoxia condition is a tissue oxygen tension of about 1% which stimulates tumor cells to produce HIF-1 $\alpha$  which in turn induces both the production of GLUT-1 and VEGF; the first increase glucose uptake and cell metabolism whilst the second favors neoangiogenesis.

Moreover GLUT-1 is able to increase the expression of adhesion molecules such as integrin  $\beta$ 1 and focal adhesion kinase (FAK) favoring cancer cell migration.

GLUT-1 is a prognostic factor that affects tumor aggressiveness and is used as a marker of staging in novel diagnostic techniques.<sup>65</sup>

### In human studies

In resected lung cancer, GLUT expression has been reported to be correlated with factors such as Ki-67 and *KRAS* mutation and did not correlate with p53 or VEGF in untreated primary lung cancer.<sup>49,66,67</sup> The VEGF and Ki-67 have a prognostic factor in surgically-treated lung cancer.<sup>49</sup> GLUT proteins not only play a role in tumor cell metabolism but also may have an effect on oncogenic factors such as *KRAS* mutation which has been found to be associated with the overexpression of GLUT-1 in lung adenocarcinoma.

GLUT-1 protein has been shown to actively interact with the immune system, and notably impaired GLUT expression seems to be a consequence of CD3/CD28 stimulation of T cells in secondary lung cancer pleural effusion.<sup>67</sup> An altered glucose uptake has been linked with an impaired GLUT expression upon stimulation of T memory cells in pleural fluid. In hypoxia condition CD8+ T cell subsets from the nonmalignant group have an increased GLUT-1 level in comparison with normoxia. Another aspect is the prognostic role of GLUT in NSCLC which is indicative of a poor outcome.<sup>68</sup>

The action of GLUT is strongly activated in cases of cancer tissue hypoxia<sup>68,69</sup> and could be overcome by wild-type p53 oncosuppressor activity.<sup>69</sup>

A higher GLUT expression has been found in lung cancer metastatic sites than in primary sites in clinical studies indicating a relationship with fast growth and tumor spread.<sup>50,70</sup>

Notably GLUT-1, GLUT-3 and GLUT-5 expression levels are significantly higher in liver metastatic lung cancer compared with primary lung cancer and normal lung tissues.<sup>70</sup>

Other pathways are activated by GLUT-1 in the mechanism of tumor growth and diffusion, and it is indeed able to influence the expression of matrix metalloproteinases (MMP) in hypoxia condition.<sup>70</sup> The latter are proteinases that play a pivotal role in tissue remodeling and inflammatory processes and favoring carcinogenesis.

### In vitro and human studies

Concerning the relationship with chemotherapy, GLUT-1 expression and glucose uptake are increased in gefitinib-resistant NSCLC cells. Indeed, gefitinib treatment further decreased the number of viable cells and increased the probability of cell death in GLUT-1 knockdown cells.<sup>71</sup>

Glucose uptake was increased in resistant NSCLC cells after gefitinib treatment suggesting that glucose intake and transport may be modulated by the action of anti-EGFR drugs.

Studies carried out on lung cancer cell lines with western blot analysis revealed further important findings.

The GLUT expression may be reduced by a molecule called apigenin in lung cancer as revealed in studies carried out in animal models or in vitro.<sup>72</sup> It is a molecule able to suppress glucose intake in lung cancer cells leading to cell apoptosis.

An overexpression of GLUT-1 has been associated with major glucose uptake and SUV value together with tumor size<sup>73,74</sup> Concurrent altered regulation of GLUT-related glucose uptake and vascular endothelial growth factor (VEGF) has been found in studies carried out on cell cultures in hypoxia condition. Indeed, an upregulation of VEGF and GLUT mRNA has been observed.<sup>75,76</sup>

GLUT amplification has been associated with another factor such as sialyl Lewis x (sLe<sup>x</sup>) implicated in tumor growth, intercell crosstalk and cell proliferation<sup>77</sup> determining a high glucose metabolism inside the cell and high cell proliferation rate.<sup>77,78</sup>

An accelerated tumor cell metabolism has also been reported combined with EGFR-tyrosine kinase which favors the maintaining of aerobic glycolysis in lung adenocarcinoma cells called the Warburg effect.<sup>79</sup>

By contrast, EGFR inhibitors (TKIs) decrease lactate production and glucose consumption, decreasing glycolytic metabolites. In lung cancer with activating *EGFR* mutations several genes are upregulated by oncogenes and are correlated with metabolism, such as GLUT-1, hexokinase 2 (HK2), pyruvate kinase and lactate dehydrogenase (LDH).<sup>79–82</sup>

Other factors involved in cell metabolism favor aerobic glycolysis which is affected by tyrosine residues and can be mediated by phosphatidylinositol 3-kinase (PI3K)/mammalian target of rapamycin (mTOR).<sup>80,81</sup>

A new therapeutic agent called WZB117 able to inhibit glucose transport in human red blood cells expressing GLUT-1 leads to a decrease in GLUT-1 protein and glycolytic enzyme levels. These changes are followed by a decline in cyclin E2 and phosphorylated RB leading to cell cycle G0 arrest and necrosis.<sup>83</sup>

Among the enzymatic pathways induced, GLUT-1 stimulates the protein pyruvate kinase muscle isozyme M2 (PKM2) which is involved in cancer cells with high metabolism rate.<sup>84</sup> PKM2 is in turn responsible for mTOR inducing the Warburg effect.<sup>84–86</sup> The Warburg effect can also be induced by p53 mutation.<sup>86–88</sup> Eventually, there is an association of GLUT-1 with the grade of lung tumor differentiation and staging, notably it is highly expressed with hexokinase in lung cancer.<sup>89,90</sup> In contrast, a down-regulation of GLUT activity is carried out by c-myc and other oncoproteins.<sup>91,92</sup> Furthermore a high expression of the above is correlated with an AMP-activated protein kinase (AMPK) decreased function and an increase of cyclin E2 action favoring tumor spread. Epigenetic alterations are eventually inducers of GLUTs by causing histone post-translational modifications as indicated in the results of studies carried out in lung cancer cell lines.<sup>93,94</sup>

## Discussion

There are various signaling pathways which induce carcinogenesis and tumor growth. Lung cancer is the leading cause of cancer death and several causes are recognized among which are environmental factors and cigarette smoking.

Since it is a fast-growing tumor characterized by high morbidity and mortality, several genes, transcriptional factors and metabolic pathways are involved in its development. Both metabolic factors and molecules involved in the cell cycle are crucial in the stepwise cancer evolution.

The two key factors considered in the present review are able to interact with pro- and anti-apoptotic molecules and can also interact with growth and transcriptional factors.<sup>95</sup>

The association of glucose transporters with hypoxia inducible factor expressed in tissue with oxygen tension of 1%, in human lung cancer suggests that fast tumor growth

requires an anaerobic effective glycolysis. We also know that glycolysis may occur in both normoxia and hypoxia conditions leading to ATP production, the so-called Warburg effect.

Exogenous factors may affect tumor development such as cigarette smoke which is by far the main avoidable cause of lung cancer, the compounds of which could on their own stimulate the production of transcriptional molecules such as HIF-1. In the current review, we focused on the main role of two important factors such as GLUT and p16 acting in opposite manner.

Nicotine derivatives are mainly able to induce pathways involving HIF-1 and GLUT-1 by acting on nicotinic acetylcholine receptors.<sup>96</sup>

HIF-1 in turn is by itself able to activate downstream signals leading to angiogenesis.

Nicotine derivatives potentially can inactivate p16 by inducing cyclin D1 to stimulate G1 phase cell cycle progression.<sup>97</sup>

On the other hand, p16-INK is in turn overexpressed in malignancies and is associated with HPV infection which by its envelope oncoproteins targets the pRb gene stimulating downstream genes involved in G1/S transition. The loss of pRb leads to p16-INK upregulation.<sup>98,99</sup>

Among the HPV-derived proteins, E6 and E7 are often associated with high p16 expression and its dysregulation is favored by cigarette tobacco compounds such as benzo(a)pyrene inducing downstream activation of cyclin dependent kinase 1.<sup>100</sup>

Nicotine-derived nitrosamines are carcinogenic factors that cause p16 inactivation hindering cisplatin proapoptosis action by Bcl-2 gene induction and playing a possible role in determining chemotherapy failure.<sup>4,101</sup>

The growth and spread of malignant cancer and notably lung cancer is the result of a close relationship between protective factors such as p16 and tumor stimulating factors.

Cell cycle, apoptosis and cell metabolism are all mechanisms subject to regulation. A fast metabolism needs fast glucose uptake and intake occurring in hypoxia condition and it is followed by VEGF-induced neoangiogenesis and activation of transcriptional factors such as STAT.<sup>102</sup>

Hypoxia in turn activates SLC2A1 and A3 transcription which encodes the high affinity glucose transporters 1 and 3. At the same time it activates enzymes such as hexokinases which are involved in glycolytic pathways so that glucose is converted into pyruvate and downstream to lactic acid by the action of the enzyme LDH, ending up in the tricarboxylic acid cycle.<sup>103</sup>

These findings confirm that there is a relationship between GLUT-1 and p16, and therefore they could both be useful in determining lung cancer prognosis.

Both immunohistochemical and western blot analyses showed high expression in the nucleus which correlated with a worse prognosis in patients.<sup>104</sup>

There is also evidence that GLUT-1 was potentially useful in distinguishing benign from malignant mesothelial tumor apart from other molecules such as epithelial membrane antigen (EMA) and p53.<sup>105</sup>

In conclusion, our findings suggest that different types of environmental, genetic and metabolic factors are involved in lung tumor development and spread. A high metabolism rate was observed in tumor cells and this was associated with a high glucose intake, high aerobic and anaerobic glycolysis leading to the Warburg effect. GLUT plays a key role in cancer metabolism growth and progression. Among the isoforms only GLUT-1 has been correlated with malignant tumor spread. Several studies in vitro and in humans have highlighted the mechanism of influence of that molecule on cell cycle and tumor spread which is associated with chemoresistance and a worse prognosis of lung cancer by stimulating cancer cell metabolism and growth, inhibiting apoptosis and favoring angiogenesis.

By contrast, p16 has an opposite action, favoring cell apoptosis and binding to cyclins CDK 4 and 6 determining, eventually, the arrest of cell cycle in G0 phase inducing tumor cell apoptosis. Therefore, both p16 and GLUT1 are overexpressed in malignant lung cancer and are potential prognostic factors, available for new target therapeutics.

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## Disclosure

The authors declare that there are no conflicts of interest.

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