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Molecular mechanisms of deguelin-induced apoptosis in transformed human bronchial epithelial cells

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Abstract

Increasing evidence has demonstrated that the phosphatidylinositol-3 kinase (PI3K)/Akt signaling pathway plays an important role in cell proliferation, apoptosis, angiogenesis, adhesion, invasion, and migration, functions that are critical to cancer cell survival and metastasis. Increased expression of activated Akt has been observed in the early stages of tobacco-induced lung carcinogenesis. Moreover, blocking the PI3K/Akt pathway specifically inhibits the proliferation of non-small cell lung cancer (NSCLC) cells, indicating that the PI3K/Akt pathway is a potential target for chemoprevention and therapy in lung cancer. The aim of this work is to study the lung cancer chemopreventive potential of PI3K/Akt inhibitors using an in vitro lung carcinogenesis model. We found that genetic or pharmacologic approaches targeting the PI3K/Akt pathway inhibited the proliferation of premalignant and malignant human bronchial epithelial (HBE) cells. After screening several natural products to identify a potential lung cancer chemopreventive agent, we have found that deguelin, a rotenoid isolated from *Mundulea sericea* (Leguminosae), specifically inhibits the growth of transformed HBE and NSCLC cells by inducing cell-cycle arrest in the G2/M phase and apoptosis, with no detectable toxic effects on normal HBE cells, most likely due to the agent's ability to inhibit PI3K/Akt-mediated signaling pathways. The specific sensitivity of premalignant and malignant HBE and NSCLC cells to deguelin suggests that this drug could be clinically useful for chemoprevention in early-stage lung carcinogenesis and for therapy in confirmed lung cancer.

Keywords: Chemoprevention; Apoptosis; Phosphatidylinositol-3 kinase; Akt; Deguelin; Lung cancer

1. Introduction

Despite recent advances in radiotherapy and chemotherapy, the severe morbidity from lung cancer and the low 5-year survival rates have improved modestly and lung cancer remains the primary cause of cancer-related deaths in both men and women in the United States [1]. Cancer chemoprevention is therefore a logical strategy to help alleviate this disease. However, all trials of lung cancer chemoprevention agents performed in the United States and Europe have been unsuccessful to date [2]. Most of these trials examined the chemopreventive efficacy of retinoid-based regimens, indicating the need to develop novel chemopreventive approaches for lung cancer. Because exposure to cigarette smoke confers the greatest risk, with approximately 90% of all lung cancers occurring in smokers, smoking-cessation campaigns have been a major focus of effort to prevent the disease. However, approximately 25% of adults in the United States continue to smoke cigarettes [3]. Furthermore, the risk of lung cancer does not diminish during the first 5 years after smoking cessation and remains higher among former smokers than among people who have never smoked [3]. Additional preventive strategies for former and current smokers are therefore needed.

It is known that the metabolites of tobacco carcinogens such as 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) and polyaromatic hydrocarbons induce DNA mutations in tumor suppressor genes and oncogenes [4]. Cells with mutated DNA are normally removed via apoptosis

Abbreviations: HBE, human bronchial epithelial; IGF-1, insulin-like growth factor-1; MAPK, mitogen-activated protein kinase; MEK, MAPK/extracellular signal-regulated kinase (ERK) kinase; NHBE, normal human bronchial epithelial; NSCLC, non-small cell lung cancer; PI3K, phosphatidylinositol-3 kinase; PKB, protein kinase B; TGF, transforming growth factor; TK, tyrosine kinase

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[4]; however, if the survival pathway is active, cells with damaged DNA persist, resulting in loss of growth control and, ultimately, lung cancer formation. On the basis of this notion, we have investigated novel agents targeting signaling mechanisms that play an important role in survival of premalignant and malignant human bronchial epithelial (HBE) cells as well as lung cancer cells. This commentary presents evidence that the PI3K/Akt pathway plays a central role in the proliferation and survival of preneoplastic cells and that deguelin, a natural product, inhibits Akt activation and has potential as a novel chemopreventive agent in lung cancer.

2. Activation of the PI3K/Akt signaling pathway and biological effects of Akt activation

Growing evidence has demonstrated that a receptor signaling system mediated by receptor and nonreceptor tyrosine kinase (TK) plays a critical role in cellular proliferation and survival and in the inhibition of apoptosis [5]. Signaling by receptor TK requires ligand-induced receptor oligomerization, which results in tyrosine autophosphorylation of the receptor subunits that mediate the specific binding of cytoplasmic signaling proteins containing Src homology-2 and protein tyrosine-binding domains, such as Grb2, Shc, IRS, Src, RasGAP, SHP-1, and PI3K [6]. Strict regulation of TK activity controls cell-cycle progression and cell proliferation, differentiation, motility, and survival [5–7]. Therefore, overexpression of receptor TK, ligand, or both could lead to deregulated TK-mediated signaling, resulting in the imbalance between the rates of cell-cycle progression (cell division) and cell growth (cell mass) on the one hand and programmed cell death (apoptosis) on the other that is characteristic of all neoplasms [8]. Two distinct signal transduction pathways, PI3K/Akt and Ras/Raf/MAPK, are crucial effectors in oncogenic TK signaling [8,9]. RTKs can also activate PI3K indirectly through Ras, which can bind and activate the p110 subunit [9], indicating a cross-talk between these two signaling pathways.

The PI3K/Akt pathway has been involved in several types of carcinogenesis. Findings from in vitro models indicate that activation of the PI3K/Akt pathway is sufficient to induce malignant transformation of human cells [10,11]. PIK3CA, which encodes p110α, has been amplified in human ovarian cancer cell lines [12]. A partially transformed phenotype has been demonstrated in mammalian fibroblasts transfected with the constitutively active form of p110α [13]. Moreover, an oncogenic mutant of p85 that can, in collaboration with the v-*raf* oncogene, transform mammalian fibroblasts has been isolated [14]. The transforming activity of PI3K is associated with its ability to activate Akt (also known as protein kinase B), a cellular homologue of the viral oncogenic protein v-Akt. Akt, a subfamily of serine/threonine protein kinases, is now known to define a family of closely related, highly conserved cellular homologues consisting of Akt1/protein kinase B (PKB)α, Akt2/PKBβ, and Akt3/PKBγ [9]. Akt is activated by growth factors, adhesive interactions with int7egrins, and signals initiated by stimulation of G-protein-coupled receptor (GPCR) [15]. Akt is also activated by isoproterenol stimulation of the β3 adrenergic receptor and Ca^{2+}/calmodulin-dependent protein kinase kinase (CaMKK) in a PI3K-independent manner [16]. 3-Phosphatidylinositol-dependent protein kinase (PDK)-1 and integrin link kinase (ILK) have been found to activate Akt by phosphorylating Thr308 and Ser473, respectively [9]. PTEN regulates Akt activity by dephosphorylating the lipid product of PI3K [16]. Activated Akt promotes cell proliferation and survival by directly phosphorylating several components involved in cell-cycle and cell-death machinery, such as glycogen synthase kinase (GSK)-3, the mammalian target of rapamycin, BAD, caspase-9, human telomerase reverse transcriptase subunit β, and IκB kinases [17–22]. Akt also induces phosphorylation of FKHR – a member of the Forkhead family of transcription factors – and thus prevents its induction of several pro-apoptotic protein expression, such as BIM and FAS ligand. In addition, Akt indirectly influence cell survival by phosphorylating IκB kinase (IKK) and MDM2, affecting the level of two central regulators of cell death – nuclear factor of κB (NF-κB) and p53, and negatively regulates the expression of CDK inhibitors (CKIs), such as KIP1 (also known as p27) and WAF1 (also known as CIP1 or p21). The effects on KIP1 seem to be transcriptional and mediated by FKHR (reviewed in Ref. [9]).

3. Involvement of Akt activation in lung carcinogenesis

Activation of Akt causes malignant transformation in vitro and in vivo in mouse models of various human cancers [23–25]. We and others have previously reported evidence that Akt activation is an early event in lung carcinogenesis; expression of pAkt is increased in premalignant and malignant HBE cells [26], reactive epithelium specimens (bronchial hyperplasia and squamous metaplasia), bronchial dysplasia, and NSCLC cells [26–28]. West et al. [29] recently reported that activation of Akt is an early biochemical effect of tobacco components in normal human lung epithelial cells that is induced by nicotine and NNK in pharmacologically relevant concentrations both in vitro in normal HBE cells and small airway epithelial cells (SAECs) and in vivo in the lungs of NNK-treated mice and human smokers with lung cancers. Lung cancer cells produce insulin-like growth factor (IGF), a major survival factor, at higher levels than do normal lung cells and exhibit a mitogenic response to exogenous IGF [30,31]. Moreover, increased expressions of epidermal growth factor receptor (EGFR) and transforming growth

factor (TGF), mutations of k-*ras*, reduced expression of PTEN, and amplification of a region of chromosome 3q that includes the p110 catalytic subunit of PI3K – all of which could induce activation of PI3K/Akt pathway – have been observed in bronchial preneoplastic lesions, NSCLC, or both [32–36]. Together, these findings indicated that Akt activation is an early event in lung tumorigenesis and, therefore, development of new chemopreventive agents that suppress the PI3K/Akt pathway may abrogate lung carcinogenesis.

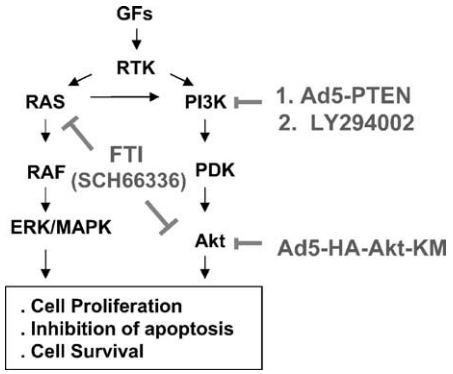

Fig. 1. Strategies to inhibit PI3K/Akt pathway; PI3K activities suppressed by LY294002 or an adenoviruses expressing PTEN (Ad5-PTEN). Akt was blocked by an adenoviruses expressing dominant negative Akt (K179M). Ras is inactivated by SCH66336, a farnesyl transferase inhibitor that was designed to inhibit Ras activation.

4. Targeting the PI3K/Akt signaling pathway in lung cancer

The effects of the PI3K/Akt pathway on the proliferation of normal (NHBE), premalignant, and malignant HBE

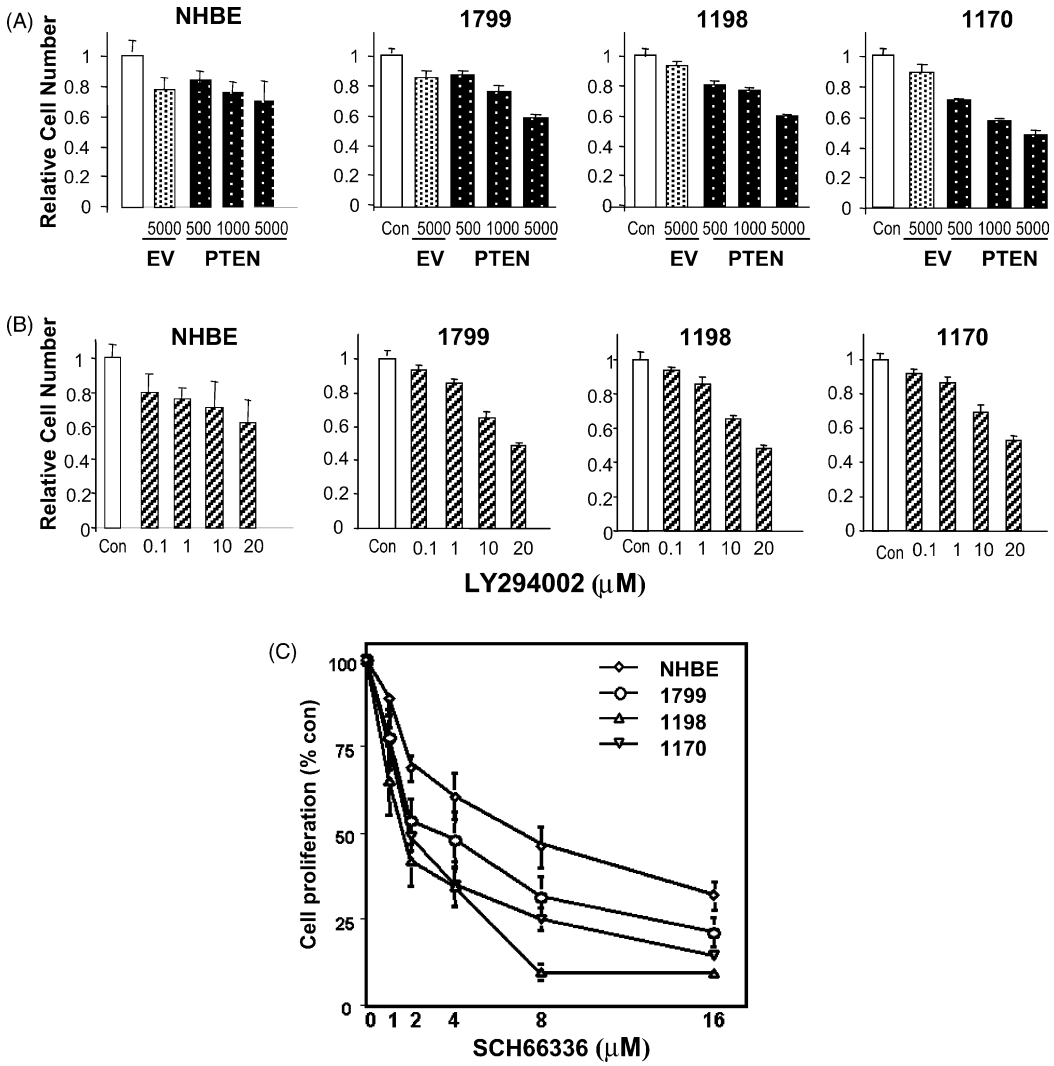

Fig. 2. Effect of the PI3K/Akt pathway on HBE cell proliferation. (B) NHBE cells and the indicated HBE cell lines were seeded in 96-well culture plates (2000–5000 cells/well), incubated with the indicated titers of (A) Ad5-CMV (EV), an empty virus, or Ad5-PTEN (PTEN) (particles/cell), (B) LY294002 (μM), or (C) SCH66336 (μM). After incubation of the cells for 3 days, they were subjected to MTT assay. Results are expressed relative to the number of cells infected with EV (A) or cells treated with medium alone (B, C). Each value is the mean (±S.D.) from five identical wells.

cells were investigated using genetic approaches with adenoviral vectors and pharmacologic approaches (Fig. 1) in normal, premalignant (1799 and 1198 cells), and malignant HBE (1170-1) cells. Specifically, the cells were treated with the PI3K inhibitor LY294002 or an adenovirus expressing either PTEN (Ad5-PTEN) [28] or dominant-negative Akt (Ad5-HA-Akt-KM) [37]. The results showed that Ad5-PTEN (Fig. 2A), LY294002 (Fig. 2B), or Ad5-HA-Akt-KM (data not shown) effectively suppressed the proliferation of 1179, 1198, and 1170-1 cells compared with NHBE cells. However, SCH66336, a farnesyl transferase inhibitor that was originally designed to inhibit Ras activation but also inhibited Akt activation [38], resulted in marginally selective inhibition of the growth of transformed HBE cells (Fig. 2C). Thus, interrupting the PI3K/Akt pathway is, in this model of lung cancer, a potentially effective chemopreventive strategy. Targeting this pathway could be extremely useful in clinical settings, especially in the treatment of NSCLC, in which constitutive activation of Akt occurs at a high frequency [39]. Moreover, manipulating Akt activity alters the sensitivity of cells to chemotherapy and irradiation [39]. Therefore, targeting Akt might enhance the efficacy of chemotherapy and radiotherapy, and increase the apoptotic potential of NSCLC cells.

5. Chemopreventive and chemotherapeutic potential of deguelin in lung cancer

Since the publication of clinical studies showing the efficacy and feasibility of chemoprevention of aerodigestive tract cancer [40], researchers have devoted much effort to the discovery and development of new chemopreventive agents. Many compounds with varied mechanisms, including retinoids, tyrosine kinase inhibitors, farnesyl transferase inhibitors, non-steroidal anti-inflammatory agents (NSAIDs), and the polyamine synthase inhibitor have been used successfully as chemopreventive agents in either animal carcinogenesis models or clinical trials [41]. However, undesirable side effects or resistance of lung cancer cells to these agents limit their long-term clinical use as chemopreventive therapy. Furthermore, all clinical trials of lung cancer chemopreventive agents performed in the United States and Europe have failed to show that such therapy benefits individuals at increased risk of developing lung cancer, thus emphasizing the need for novel lung cancer chemopreventive strategies.

Since we and others have found that one of the most promising molecules for chemoprevention and treatment of lung cancer is targeting of Akt, several natural plant products and dietary constituents have been screened to identify those with inhibitory effects on proliferation of transformed HBE cells by blocking Akt activation. Among many tested natural products, deguelin (Fig. 3), a rotenoid isolated from several plant species including *Mundulea sericea* (Leguminosae), has shown potential cancer chemopreventive activity. Deguelin effectively prevented 7,12-dimethyl(*a*)benzanthracene-induced preneoplastic lesions in mouse mammary organ culture [42]. Further, in a murine two-stage DMBA/TPA skin carcinogenesis model, treatment with topical deguelin markedly suppressed disease induction of papillomas in mice treated at a low dose (33 μg) and completely suppressed disease (i.e., no tumors in any mice) at a high dose (330 μg) [43]. The antitumor efficacy of deguelin was also shown in an NMU-induced rat mammary carcinogenesis model: although deguelin had no effect on tumor incidence, the mean tumor number was reduced from 6.8 to 3.2 tumors per rat in the group treated with high-dose deguelin [43].

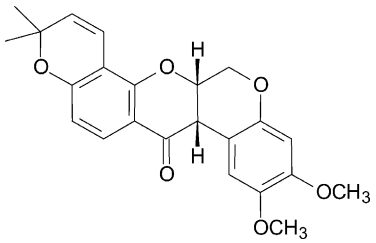

Fig. 3. Structure of deguelin.

We have demonstrated that deguelin has potent apoptotic activities in transformed HBE cells and a variety of NSCLC cell lines at doses attainable in vivo, with minimal effects on NHBE viability [44]. Deguelin treatment induced both cell-cycle arrest in the G2/M phase and apoptosis [26,44]. In addition, deguelin inhibited PI3K activity and reduced pAkt levels and activity but had minimal effects on the MAPK pathway; furthermore, overexpression of a constitutively active Akt blocked deguelin-induced growth arrest and apoptosis [44]. These findings indicate that the proapoptotic activity of deguelin results from its ability to inhibit PI3K/Akt-mediated signaling pathways. In addition, deguelin induced a partial and delayed inhibition of PI3K activity, compared with stronger, earlier inhibition of Akt activity, suggesting that more than one mechanism mediates deguelin-induced suppression of Akt activity. Deguelin also inhibits the expression of cyclooxygenase (COX)-2 [44], which is largely regulated by Akt activity [45], without affecting the COX-1 protein level. COX catalyzes the conversion of arachidonic acid to prostaglandins, which can cause tumor cell growth, suppress immune surveillance [46], and induce metabolic activation of the tobacco components PAH and NNK [47]. Other PI3K/Akt inhibitors, such as LY294002 and farnesyltransferase inhibitor SCH66336 [38], were less effective than deguelin at inhibiting the growth of premalignant HBE cells (unpublished data); LY294002 and SCH66336 required more than 10 and 1 μM, respectively, to induce detectable growth inhibition in premalignant and malignant HBE cells. The difference in the effects on COX-2 expression between deguelin and these PI3K/Akt inhibitors warrants further investigation.

All these findings indicate that deguelin is a potentially useful chemopreventive agent in lung cancer. In addition, since malignant HBE cells and NSCLC cell lines are also sensitive to deguelin, this proapoptotic drug has potential as a therapeutic agent against lung cancer.

6. Conclusion

The evidence presented herein suggests that Akt is activated at an early stage of lung carcinogenesis through a variety of mechanisms. Because Akt activity is crucial for cell proliferation and survival of transformed HBE and NSCLC cells, Akt is likely to be an important factor in early progression of lung carcinoma. Interestingly, activation of Akt is an early biochemical effect of tobacco components on human lung epithelial cells in vitro and in vivo [36]. Thus, suppression of Akt activation could be an effective preventive strategy, especially for smokers. The potential of inhibitors of PI3K/Akt activities for treatment of lung cancer should also be considered.

Given this evidence of Akt's involvement in lung cancer and the fact that this malignancy is the leading cause of cancer death worldwide, the need to develop small molecules such as deguelin that target Akt activation is urgent. The present data provide evidence that deguelin selectively inhibits the proliferation of transformed HBE cells by blocking Akt activation and that inhibition of Akt by deguelin is the mechanism that mediates its apoptotic effects in transformed HBE cells. Because Akt activity alters the sensitivity of NSCLC cells to chemotherapeutic agents and irradiation [39], treatment with deguelin may enhance the efficacy of chemotherapy and radiotherapy, and increase the apoptotic potential of NSCLC cells.

The potential of deguelin as a chemopreventive and chemotherapeutic agent in lung cancer has recently attracted much attention. Before this approach is used clinically, more experimental studies, especially in animal models, are needed to provide in vivo evidence of efficacy in preventing early disease progression and potential to increase the effectiveness of current chemotherapy and radiation therapy. Since rotenoids inhibit NADH:ubiquinone oxidoreductase activity, an enzyme complex in mitochondrial oxidative phosphorylation, which is associated with cardiotoxicity, respiratory depression, and nerve conduction blockade at high doses ($LD_{50} = 10–100$ g in humans) [42,43], additional studies are needed to evaluate any potential systemic toxicity of deguelin. In addition, further studies to define the relation between structure and activity of analogues of the rotenoids are warranted.

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