

Extracellular Matrix and Aberrant Signaling in Lung Carcinoma: Role of Fibronectin in the Control of Human Lung Carcinoma Cell Growth, Apoptosis and Resistance to Therapy

ShouWei Han^{1,*} and Jesse Roman^{1,2,*}

¹Department of Medicine, Division of Pulmonary, Allergy and Critical Care Medicine, Emory University School of Medicine, Atlanta, Georgia 30322, USA; ²Veterans Affairs Medical Center, Atlanta, Georgia 30033, USA

Abstract: Despite recent advances in understanding the molecular biology of lung carcinoma and the introduction of multiple new chemotherapeutic agents for its treatment, its dismal five-year survival rate (<15%) has not changed substantially. The lack of advancements in this area reflects the limited knowledge available concerning the factors that promote oncogenic transformation and proliferation of carcinoma cells in the lung. Tumor growth and invasion are not only the result of malignant transformation, but also depend on environmental influences from their surrounding stroma, local growth factors, and systemic hormones. In particular, the composition of the extracellular matrix is believed to affect malignant behavior *in vivo*. This document reviews information that implicates the matrix glycoprotein fibronectin in regulation of lung carcinoma cell proliferation, apoptosis and resistance to therapy. Fibronectin is highly expressed in chronic lung disorders where most lung carcinomas are identified. Data available to date indicate that by binding to specific integrin receptors expressed on tumor cells, fibronectin stimulates a number of intracellular signals implicated in the pathobiology of lung carcinogenesis including GTPases, mitogen-activated protein kinases, and the PI3-Kinase/Akt/mTOR pathway. Targeting fibronectin and integrin-mediated signals in tumor cells represents a promising target for the development of effective anti-cancer strategies.

Key Words: Fibronectin, integrin, signaling, human lung carcinoma cells, proliferation, therapy.

INTRODUCTION

New approaches for the treatment and prevention of lung carcinoma are clearly indicated, but depend greatly on a better understanding of the cellular and molecular mechanisms that control oncogenesis and tumor growth in the lung. Tumor growth and invasion are not only the result of malignant transformation, but are also dependent on environmental influences from the surrounding stroma, local growth factors, and systemic hormones [1, 2]. While most studies directed at elucidating the factors that control lung carcinogenesis focus on genetic mutations, dysregulated growth factor production, and aberrant signaling, little is known about the impact of the lung stroma on lung tumor development and progression.

Many cell types (e.g., epithelial cells, fibroblasts) are dependent upon adhesion to the extracellular matrix for their continued survival, and undergo apoptosis upon detachment from the matrix [3]. Although transformed cells are characterized by their ability to grow in the absence of cell adhesion to a matrix, solid tumor cells exist *in vivo* submerged in a matrix-rich environment, and they are in a state of dynamic interplay between anchorage dependence and independence. Therefore, the interactions between carcinoma cells and their surrounding stroma are likely to modulate key processes in tumor cells such as proliferation and apoptosis [3-6].

This document reviews information that links aberrant extracellular matrix composition in lung with the development and progression of lung carcinoma. It is not meant to provide a comprehensive summary of the proposed roles of lung extracellular matrices (and their receptors) on lung carcinoma. Instead, it focuses on *fibronectin*, a matrix glycoprotein highly expressed in acute and chronic forms of lung disease that has been implicated in the biology of cancer. The document describes the receptors that mediate recognition of fibronectin in lung and the intracellular signals elicited through fibronectin receptor binding that promote lung carcinoma growth and survival. Most importantly, the document explores how fibronectin influences established and recently described pro-oncogenic pathways implicated in the pathogenesis of lung carcinoma. The implications of this new information for the development of novel strategies targeting fibronectin-mediated signals in lung carcinoma cells are also reviewed in the hope of stimulating further research in this relatively unexplored area of investigation.

FIBRONECTIN IN LUNG

Over 90% of patients diagnosed with lung cancer suffer from chronic lung diseases that are idiopathic or related to exposure to environmental hazards [7]. With regards to the latter, tobacco consumption is the most important risk factor for the development of lung carcinoma [8]. However, patients who may or may not smoke, but who suffer from emphysema, idiopathic pulmonary fibrosis, asbestosis and other chronic lung diseases are also at increased risk of developing lung carcinoma [9, 10]. Furthermore, lung cancer may develop years after smoking cessation in patients with tobacco-related lung disease [11]. These observations suggest that there are structural and/or functional alterations in the lung

*Address correspondence to these authors at the Division of Pulmonary, Allergy and Critical Care Medicine, Emory University School of Medicine, Whitehead Bioresearch Building, 615 Michael Street, Suite 205-M, Atlanta, Georgia, 30322, USA; Tel: +1 404-712-2970; Fax: +1 404-712-2974; E-mail: shan2@emory.edu; jroman@emory.edu

of affected individuals that promote carcinogenesis. One characteristic shared by these chronic lung conditions relates to dramatic alterations in lung architecture caused by aberrant lung connective tissue content and composition [12, 13]. Thus, it is proposed that, together with other factors, the abnormal deposition of extracellular matrices in lung renders the host susceptible to the development of carcinoma.

Alterations in the expression, content, and turnover of collagens, proteoglycans, basement membrane components like laminins and elastin, among others, have been documented in chronic lung disorders [12-15]. Another matrix component that is altered in chronic lung disease is *fibronectin*. This glycoprotein has been implicated in many physiological and pathological processes including development, tissue repair, angiogenesis, thrombosis, inflammation, and oncogenesis [16-19]. These observations, coupled with studies showing that environmental agents linked to the development of chronic lung disease and cancer (e.g., tobacco and mineral dusts) [20, 21] stimulate fibronectin expression in lung both *in vitro* and *in vivo*, further implicate this molecule in lung carcinoma development and progression.

Fibronectin is a heterodimeric extracellular matrix glycoprotein that consists of two monomers, each roughly of 250 kD, which are joined by disulfide bonds at their carboxyterminal end. These monomers are highly modular proteins comprised of groups of repeated homology units of type I, II, and III repeats [16, 22] (Fig. 1A). Fibronectin can be found in over 20 forms all generated from the variable splicing of the pre-mRNA of a single gene. Alternative splicing can

occur at three sites: the Extra Type III Domain A (EIIIA or EDA), the Extra Type III Domain B (EIIIB or EDB), and a V (for variable) region. The biological roles of these variants remain largely unexplored, but there is evidence implicating them in oncogenesis (see later).

Fibronectin is highly expressed in embryonic organs, but after birth, its expression is limited to the liver (also called *plasma* fibronectin), alveolar macrophages and large vessels, among few other tissues [23]. However, after lung injury, the expression of *cellular* fibronectin is increased [16, 17]. Of particular interest is the fact that essentially all forms of acute and chronic lung injury in both animals and humans are associated with increased fibronectin expression. Under these circumstances, fibronectin originates in bronchial and alveolar epithelial cells, interstitial fibroblasts, and interstitial and alveolar macrophages. This is the reason why fibronectin expression is considered an early marker of lung injury [16, 17].

The study of this molecule has been complicated by the fact that knockout mutations of fibronectin lead to early embryonic lethality [24]. Nevertheless, its functions have been surmised through many studies *in vitro* revealing the effects of fibronectin in diverse cell types. Fibronectin promotes the proliferation of fibroblasts, aids the deposition of collagen, stimulates the chemotaxis of monocytes and other immune cells, promotes the expression of proteases, and induces cytokine production in macrophages [25-31]. Some of these effects have been linked to its ability to induce a number of transcription factors including AP-1 and NF- κ B [32]. Fibro-

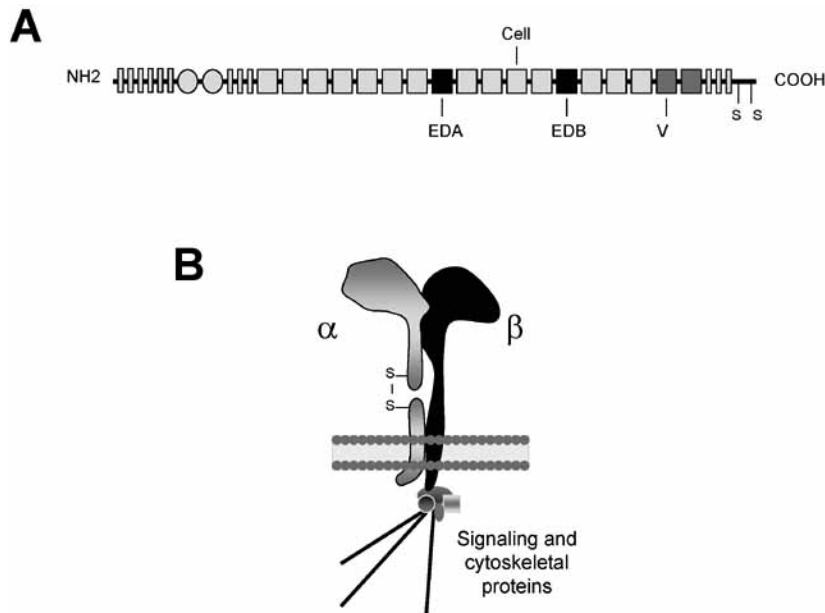


Fig. (1). Fibronectin and integrin receptors.

A, Fibronectin domain structure. This is a schematic representation of a fibronectin monomer; only one subunit is presented for clarity. Fibronectin is composed of several groups of homologous repeats. The main splicing sites are depicted (EDA, EDB and V). The arginine-glycine-aspartic acid (RGD) sequence that mediates binding to the α 5 β 1 integrin is located in the mid-portion of the molecule (Cell)

B, Schematic representation of integrin. Integrins are heterodimeric receptors composed of α and β subunits assembled non-covalently at the cell surface. Each subunit has a long extracellular domain that interacts with ligand and an intracellular domain that interacts with intracellular 'adapter' molecules (e.g., Grb2, Crk), cytoskeletal proteins, and signaling molecules.

nectin and its fragments are also known to stimulate the expression of matrix metalloproteinases implicated in cancer metastasis [33, 34].

Fibronectin exerts its effects through the binding of integrin receptors expressed in essentially all lung cell types [35-37]. Integrins are a family of heterodimeric receptors made of α and β subunits that assemble at the cell surface in a non-covalent fashion. They are characterized by a large extracellular ligand binding domain and a short intracellular domain connected by a single chain transmembrane domain (Fig. 1B). The specificity of each integrin for its ligand(s) depends on the $\alpha 5$ subunit composition. Integrin binding results in the activation of a number of intracellular signals including the phosphorylation and activation of protein kinases (e.g., focal adhesion kinase), influx of calcium ions, and changes in intracellular pH [38].

Several integrins mediate cell-cell interactions, whereas others mediate binding to the extracellular matrix. In the latter group, members of the $\beta 1$ family of integrins are the best described and include receptors for collagens, laminin and fibronectin. Within the $\beta 1$ integrin subfamily, the integrin $\alpha 5\beta 1$ is considered to mediate many of the biological effects of fibronectin. As was seen for fibronectin, the study of $\alpha 5\beta 1$ has also been hampered by the fact that knockout mutations of the $\alpha 5$ integrin subunit are also embryonic lethal, again highlighting the critical role of the fibronectin $\alpha 5\beta 1$ pair in embryogenesis [39]. *In vitro*, blockade of $\alpha 5\beta 1$ inhibits fibroblast adhesion, migration and proliferation [40]. Although other integrins interact with fibronectin [e.g., $\alpha v\beta 6$, $\alpha 4\beta 1$], it is $\alpha 5\beta 1$ that mediates many of the pro-inflammatory effects of fibronectin and aids significantly in the incorporation of its ligand into the matrix [41, 42].

FIBRONECTIN AND INTEGRINS IN LUNG CARCINOMA

Alterations in the expression of fibronectin and integrins in cancer cells have long been described in the literature [43-47]. Studies of solid human tumors show that among the early signs of malignant transformation is the fragmentation of pericellular fibronectin that is concomitant with an increase in fibronectin deposition in the peritumoral stroma [18]. In lung carcinoma, fibronectin expression is altered, especially in non small cell lung carcinoma cells (NSCLC) [48, 49]. Alterations in the relative expression of specific fibronectin isoforms have also been identified in cancer. The fibronectin EDB isoform is expressed in fetal and neoplastic tissues and is generally absent in their normal counterparts. EDB fibronectin has also been found to be present almost exclusively in the modified extracellular matrix surrounding newly-formed blood vessels in tumors, while being absent from the normal vasculature in adult organs [50]. Similarly, others have postulated that the expression of EDB fibronectin might have diagnostic value in patients with head and neck squamous cell carcinoma [51].

In certain tumors, overexpression of the integrin $\alpha 5\beta 1$ is associated with a more malignant phenotype [52]. This integrin is generally not found in normal lung tissue, but it is expressed in a considerable fraction of lung carcinomas; this is associated with worse survival [53]. The expression of $\alpha 5\beta 1$ in NSCLC correlates with tumor progression, and its

overexpression is associated with decreased survival [54]. In an immunohistochemical study of NSCLC tissues from patients, a correlation was found between overexpression of $\alpha 5\beta 1$ (and lost of expression of collagen) and lymph node metastases [54]. Increased expression of integrin subunits is also considered a poor prognostic factor in small cell lung carcinoma (SCLC) [55].

Together, these observations strongly implicate fibronectin and fibronectin integrin receptors in the pathogenesis of lung carcinoma, and have sparked interest in the mechanisms by which these molecules exert their effects. Among these, several studies have found that fibronectin and other extracellular matrices stimulate angiogenesis to supply nutrition of growing tumors, and induce matrix degrading proteases capable of facilitating metastasis [34, 56, 57]. This review focuses on studies exploring the intracellular signals that mediate the pro-oncogenic effects of fibronectin in lung carcinoma cells.

ABERRANT SIGNALING IN LUNG CARCINOMA

Several studies have implicated aberrant intracellular signaling in the development of lung carcinoma. Mutations in ras, c-myc, p53 and other genes, for example, are considered to play key roles in the process of carcinogenesis [58-60]. It is generally believed that aberrant signaling leading to lung carcinoma is due to the interplay between internal factors (e.g., genetic mutations) and external factors (e.g., environmental hazards) that cause DNA damage (e.g., DNA adducts) and promote the activation of pro-oncogenic pathways, while silencing anti-tumor or pro-apoptotic pathways. Here, it is proposed that aberrant signaling can be induced or amplified by the extracellular milieu that surrounds lung carcinoma cells. This is based on information demonstrating that integrin activation can induce signals that affect cell cycle control and survival (see below); in essence, matrix binding to tumor integrins can stimulate aberrant signaling leading to carcinoma growth and resistance to therapy.

Mounting evidence demonstrates the activation of several intracellular signaling pathways implicated in lung carcinogenesis in response to fibronectin. This is true for the mitogen-activated protein kinase kinase (MEK1)/ extracellular regulated kinase (Erk) family, a dual-specificity protein kinase family that controls cell growth and differentiation, and that is activated by a wide variety of extracellular signals including growth and neurotrophic factors, cytokines, hormones, and neurotransmitters [61, 62, 63]. Signaling through the Erk pathway promotes cell immortalization *via* telomerase induction, stimulates growth factor-independent proliferation and insensitivity to growth-inhibitory signals by cell cycle activation, promotes autocrine signaling and inactivation of tumor suppressor genes, and enhances invasion and metastases *via* stimulation of cellular motility and extracellular matrix remodeling. We and others have demonstrated that fibronectin, by binding to its $\alpha 5\beta 1$ integrin receptors, stimulates human cancer cell growth *in vitro*, at least in part, through activation of MEK1/Erk [64-66]. Interestingly, blockade of the interaction between fibronectin and $\alpha 5\beta 1$ integrin can activate or inhibit MEK1/Erk signals depending upon the reagents used and the cells studied [67-70]. Fibronectin fragments may promote human cell adhesion and pro-

liferation through MEK-1/Erk activation as demonstrated in non-tumor cells including retinal endothelial cells, thyroid cells, and monocytic cells [68]. However, it has been reported that anastellin, a fragment of the first type III repeat of fibronectin, inhibits Erk activation and causes G₁ arrest in human microvessel endothelial cells suggesting that the antiangiogenic properties of anastellin observed in mouse models of human cancer may be due to its ability to block endothelial cell proliferation by modulating Erk signaling pathways and down-regulating cell cycle regulatory gene expression required for G₁-S phase progression [71]. Thus, intact fibronectin and distinct fibronectin fragments may affect tumor cells differently.

Fibronectin can also stimulate the family of small GTPases. This is important in view that this family has been implicated in many cellular functions such as cell adhesion, cell motility and migration, growth control, and cell contraction [72, 73]. In tumors, the small GTPase RhoC enhances metastasis when overexpressed, whereas a dominant-negative Rho inhibits metastasis. Analysis of the phenotype of cells expressing dominant-negative Rho or RhoC indicates that RhoC is important in tumor cell invasion [74]. Rho GTPases are overexpressed in human breast cancer cells and are involved in a variety of cellular processes including cell-cell contact and malignant transformation [75]. Integrin-mediated cell adhesion activates members of the small GTPase family including RhoA, Rac1, and CDC42, which regulate actin cytoskeletal organization, lead to the turnover of stress fiber/focal adhesions, and influence the formation of lamellipodia and filopodia [76]. Based on this information, one might predict a role for small GTPases in mediating some of the effects of fibronectin in tumor cells. This has been documented in studies showing that fibronectin binding to β 1 integrins is necessary for both the phagokinetic motility and transcellular migration of rat ascites hepatoma cells through the activation of Rho-ROCK (Rho-kinase) [77]. In NIH3T3 fibroblasts, cell adhesion to fibronectin leads to rapid activation of the Rho family GTPases Cdc42 and Rac, which contributes to cell spreading [78]. Rho is involved in the stimulation of cyclin D1 protein accumulation by fibronectin and NIH 3T3 cell adhesion to fibronectin is required to suppress p21^{Cip/Waf} RNA levels, a process which requires Rho activity [79, 80]. We recently demonstrated that fibronectin activates the Rho signal pathway in NSCLC cells [81]. Specifically, we reported that fibronectin stimulated lung carcinoma cell proliferation by reducing the expression of p21, while inducing Cyclin D1. The reduction of p21 in our system appeared to be mediated through Erk activation and Rho-kinase signaling leading to alterations in p21 gene transcription.

Protein kinases A and C are important for regulating cell recognition of extracellular matrices by integrins and integrin-mediated signaling. Inhibitors of protein kinases A and C impair the adhesion and migration of hepatocellular carcinoma cells induced by prostaglandin E₂ [82]. This may have important implications for lung cancer since activation of these kinase pathways might promote tumor cell recognition of fibronectin. Consistent with this, it has been found that the direct activation of protein kinase C increases the binding activity of β 1-integrins resulting in increased adhesion of SCLC cells and histiocytic lymphoma cells to matrix pro-

teins including fibronectin [83, 84]. In Lewis Lung Carcinoma (LLC) tumors, protein kinase A signaling modulates adhesion to or migration through fibronectin, and facilitates tumor transit from the primary tumor site [85]. Others have shown that prostaglandin E₂ accelerates ProNectin F (TM) (a proteolytic fragment of fibronectin) mediated adhesion in mouse mastocytoma P-815 cells, and this was blocked by the pharmacological inhibitor of protein kinase A, H-89, indicating that protein kinase A activity is a critical mediator of the prostaglandin E₂ effect in these cells [86]. In addition to a role in cellular recognition of fibronectin, these protein kinases seem to mediate many of the matrix-related signals activated through integrin binding. In support of this idea, fibronectin was shown to stimulate human lung carcinoma cell proliferation and diminish apoptosis by inducing cyclooxygenase-2 (COX-2) gene expression and prostaglandin E₂ biosynthesis partly through activation of protein kinase C [64].

Cell adhesion to fibronectin also results in the activation of phosphatidylinositol 3'-kinase (PI3-K)/Akt signals [87, 88]. It has been speculated that stimulation of PI3-K/Akt activity by integrin-mediated cell adhesion may regulate anchorage-dependent cell cycle progression. Of note, overexpression of this proto-oncogene pathway has been detected in several human cancers [89, 90]. In SCLC, fibronectin leads to increased viability and changes in cytoskeletal functions that are partially mediated through the PI3-K pathway [91]. Others have shown that inhibition of the PI3-K/Akt signaling pathway may sensitize lung cancer cells to the action of commonly used anticancer drugs [92]. Interestingly, the tumor suppressor gene PTEN, a phosphatase that counteracts PI3-K to decrease PI3-K/Akt activation, is deleted in many human cancers [93, 94].

The above observations regarding the PI3-K/Akt pathway are intriguing in view of new information implicating signaling *via* the mammalian target of rapamycin (mTOR) pathway in cell cycle progression and cell proliferation. This pathway is induced through PI3-K and Akt activation, and is frequently found upregulated in human cancers [95, 96]. The mTOR pathway, with downstream activation of its target p70S6K, is considered a novel therapeutic target [97], and the link between this pathway and fibronectin has been recently reported. Rapamycin, an inhibitor of the mTOR pathway, has been shown to inhibit fibronectin-induced smooth muscle cell migration through a pathway that involves at least α v β 3-integrin, PI3-K, mTOR, and p70S6K [98]. We recently found that fibronectin stimulates the phosphorylation of Akt and of p70S6K1, which suggests activation of the mTOR pathway in NSCLC cells. Blockade of the Akt signal resulted in the elimination of fibronectin-induced p70S6K phosphorylation and in inhibition of NSCLC cell growth indicating a key role for PI3-K/Akt/mTOR/p70S6K signaling in fibronectin-induced NSCLC cell growth [99].

Although the data presented above refer mainly to the effects of fibronectin on tumor growth and progression, there are data implicating tumor cell-stromal interactions in cancer resistance to chemotherapy and radiation. Several studies have shown that cancer cells, including SCLC cells, are protected from apoptosis induced by various anti-cancer drugs when the cells are plated on extracellular matrix proteins

[100]. Similarly, in hepatoma cells, $\beta 1$ integrin-mediated signaling contributed to resistance to chemotherapy [101]. Disruption of integrin-mediated cell-matrix interactions induces apoptosis in pancreatic cancer cells [102]. Other studies suggest that tumor-matrix interactions may be critical for the emergence of drug-resistance in certain tumor populations leading to treatment failure in leukemias [103]. The mechanisms responsible for the ability of extracellular matrices to promote tumor cell survival and resistance to therapy remain unelucidated, but activation of integrins through tyrosine phosphorylation and induction of integrin clustering have been demonstrated in cultured human A549 lung cancer cells irradiated with 0-8 Gy or treated with cisplatin, paclitaxel, or mitomycin. Under these circumstances, adhesion to fibronectin significantly reduced radio- and chemosensitivity [104]. In other work, fibronectin enhanced the viability of a SCLC cell line, and this appeared associated with phosphorylation of p125FAK and alterations in cytoskeletal function [105]. Using A549 human lung cancer cells and CCD32 normal human lung fibroblasts, others found that cells exposed to fibronectin showed significantly greater survival compared to control cells when irradiated [88]. There was also a significantly greater elevation of G2/M cells in fibronectin cultures after irradiation [88].

In summary, the relative concentration of fibronectin in lung and its integrin-mediated recognition by tumor cells are likely to affect the overall proliferation and survival of lung carcinoma cells and their resistance to anti-cancer drugs.

However, there are several aspects of this information that need to be highlighted. First, it appears that fibronectin-induced signals are transmitted through redundant pathways (Fig. 2). Therefore, targeting any particular pathway might not be sufficient to block the mitogenic effects of fibronectin on lung carcinoma cells. Instead, strategies will need to focus on preventing fibronectin recognition and its binding to tumor cell integrins (see later). Second, fibronectin should not be viewed solely as a host factor that regulates tumor behavior since it is also produced by tumor cells and can therefore act as an autocrine factor. Tumor-derived fibronectin is assumed to have similar effects than host-derived fibronectin since inhibition of fibronectin and $\alpha 5 \beta 1$ expression in NSCLC cells by siRNA technology reduces tumor cell proliferation (Han and Roman, unpublished observations). One caveat is that tumor cells might produce different fibronectin isoforms that have autocrine effects that differ from those produced by host cells. Consequently, it appears that cancer cells can create a specialized environment as a consequence of autocrine and paracrine effects that, using an analogy with inflammation proposed by others, “likens lung carcinoma to a ‘wounding reaction’ with the laying down and remodeling of the extracellular matrix” [100]. Also, modulation of fibronectin recognition is likely to promote tumor growth and survival. Considering this, it is intriguing to note that alternatively spliced variants of integrin subunits, which affect intracellular signaling pathways differentially, have been identified in prostate and breast carcinomas [106].

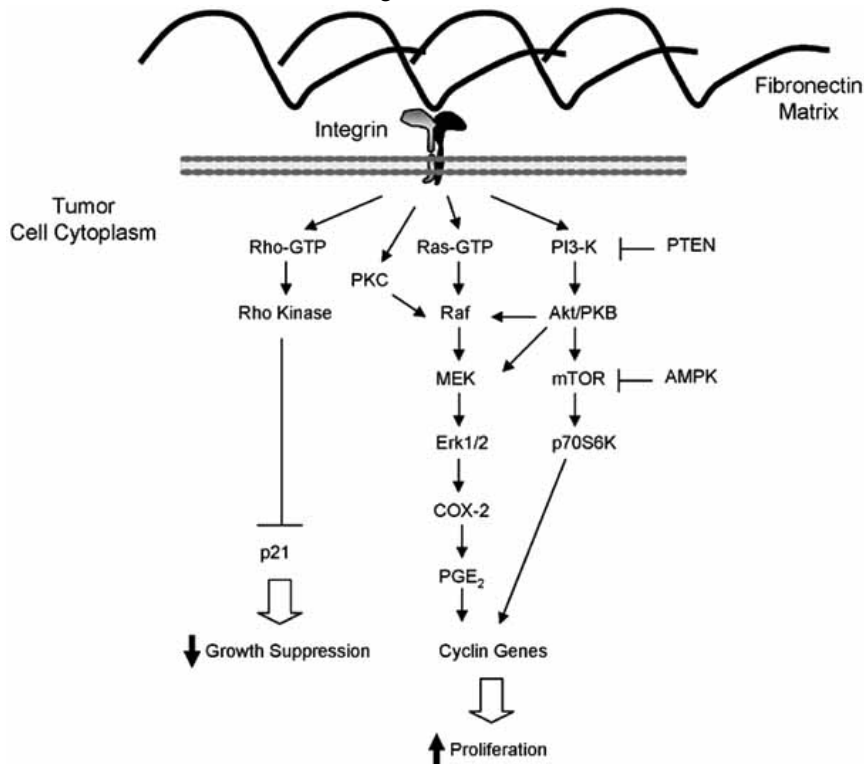


Fig. (2). Signal transduction through $\alpha 5 \beta 1$ integrin in lung carcinoma cells.

Ligand binding to $\alpha 5 \beta 1$ in lung carcinoma cells seems to trigger a number of redundant signaling events that include activation of Rho kinase, protein kinase C, the Ras and MEK/Erk pathway, as well as the PI3-K/Akt/mTOR/p70S6K pathway. These events culminate in the expression of cell cycle control genes (e.g., Cyclin D1) and inhibition of tumor suppressor genes like p21, which lead to tumor cell proliferation.

IMPLICATIONS FOR UNDERSTANDING CARCINOMA CELL BIOLOGY AND FOR DRUG DEVELOPMENT

The observations described above strongly implicate fibronectin and its receptors in the pathobiology of lung carcinoma. However, one must be cautious when extrapolating these studies (many of which were performed in cultured cells) to the clinical situation. Also, it is important to note that, despite the evidence presented above, a controversy remains regarding the true role of fibronectin and its receptors in cancer development and progression. Some studies suggest that malignant potential and metastatic ability correlate with a decrease (not an increase) in fibronectin expression [107]. Others have shown that oncogenic transformation is associated with a decrease in the expression of fibronectin and several integrins including $\alpha 5 \beta 1$ [108]. Similarly, others have suggested that tumor cells overexpressing $\alpha 5 \beta 1$ are less tumorigenic than their parent cells [109]. To test the role of these molecules in the context of spontaneous tumor formation, Taverna *et al.* [110] recently analyzed tumor development in mice genetically altered in the genes for fibronectin or $\alpha 5$ integrin. They found that heterozygosity for either gene does not lead to an increased incidence of tumors, alteration in tumor spectrum, or increased levels of metastasis, even when the fibronectin or $\alpha 5$ mutations were combined with mutations in the p53 tumor suppressor gene that lead to spontaneous tumor formation. In their study, loss of heterozygosity for $\alpha 5$ did not correlate well with tumorigenesis or metastasis. Moreover, chimeric animals containing high proportions of $\alpha 5$ -null cells did not show an increased incidence of tumors or a change in tumor progression.

Although the above studies argue against a role for fibronectin in tumor development, it must be emphasized that further investigations will be required in more relevant models of lung carcinogenesis and in clinical studies before conclusions can be drawn regarding human disease. In particular, these discordant findings need to be reconciled before applying anti-cancer strategies that target fibronectin and/or its integrin receptors. It is possible that fibronectin and $\alpha 5 \beta 1$ play different roles in malignant transformation, growth and survival, tissue invasion, and metastases, processes that are linked mechanistically, but that are clearly distinct from one another. Fibronectin may not be involved in the initial oncogenic transformation of cells, but may promote tumor growth and survival. Integrin-mediated signals might promote carcinoma progression by inducing intracellular signals that promote the expression of genes involved in cell cycle progression (e.g., cyclin D1) and by inhibiting apoptosis (e.g., bcl-2), while inhibiting genes involved in tumor suppression (e.g., p21). This process might be amplified by the ability of this integrin to promote fibronectin matrix assembly, thereby facilitating the deposition of fibronectin around tumor cells. On the other hand, a decrease in the expression of fibronectin and/or integrin-related adhesion might be required to promote detachment of tumor cells from their substrate in order to allow their migration and invasion of surrounding tissues thereby enhancing progression and metastases. Considering these two scenarios, one would predict that the effectiveness of anti-fibronectin or anti-integrin strategies in

the management of lung cancer might depend on the staging of the tumor and on its invasive potential, among other factors.

Regardless of this controversy, studies directed towards the development of strategies against fibronectin and/or its integrin receptors have been reported. Antagonists of integrins have been shown to suppress cell migration and invasion of primary and transformed cells, and to induce apoptosis of primary tumor cells [111]. In animal models of cancer, antibodies and peptide antagonists of integrins $\alpha v \beta 3$ and $\beta 1$ inhibit angiogenesis, tumor growth and tumor metastasis [112-117]. Currently, humanized antibody antagonists of $\alpha 5 \beta 1$ as well as cyclic peptide inhibitors of integrins $\alpha v \beta 3$ and $\alpha 5 \beta 1$ are under investigation as angiogenesis-inhibiting therapies in cancer clinical trials [111]. Thus, strategies based on blocking $\alpha 5 \beta 1$ integrin-mediated adhesion and/or survival signals may represent a new therapeutic approach to improve the response to chemotherapy in lung cancer. Specific fibronectin isoforms (e.g., EDB) might become yet another target for the development of more specialized anti-cancer strategies, but the fact that there are many different isoforms whose functions are largely unknown will undoubtedly hinder this effort [118]. Lately, there has been increased interest in cancer-associated fibroblasts as novel targets for therapy since these cells are believed to assist in the generation of a protective environment for tumor cells [119].

Other more global strategies might target fibronectin and integrin-mediated signals indirectly. COX-2 inhibitors are an example of this. COX-2 is an inducible form of cyclooxygenase that represents a potential pharmacologic target to prevent and treat a variety of malignancies [120]. Overexpression of COX-2 is considered important for tumor invasion, angiogenesis, resistance to apoptosis, and suppression of host immunity [121]. COX-2 is frequently elevated in human NSCLC and increased COX-2 expression leads to increased invasion by NSCLC cells [122, 123]. An association between fibronectin and upregulation of COX-2 and its downstream signals has been reported in several studies [34, 124, 125]. We have reported that fibronectin stimulates lung carcinoma cell proliferation *in vitro* through increased COX-2 gene expression and PGE₂ secretion [64]. Therefore, targeting COX-2 is likely to inhibit pro-oncogenic signals elicited by fibronectin. Consistent with this, we showed that the selective COX-2 inhibitors NS398 and Nimesulide inhibited fibronectin-induced NSCLC cell proliferation [64]. Equally interesting is the observation that COX-2 inhibitors may affect other aspects of this system since they reduce the mRNA expression and protein production of the integrin $\alpha 5$ subunit, and decrease tumor cell adhesion to fibronectin [126]. Similar observations have been made in other systems [127-129].

Another example of global anti-cancer strategies capable of affecting fibronectin and integrins are the endogenous and exogenous agonists for peroxisome proliferator activated receptors (PPARs). PPARs are members of the steroid-thyroid hormone superfamily of ligand-activated transcription factors [130]. PPARs, like other hormone nuclear receptors, heterodimerize with the retinoid X receptor (RXR) and bind to specific DNA response elements termed DR-1, which consists of a direct repeat of two AGGTCA half-sites

separated by a single intervening nucleotide. Of the three PPAR isoforms identified, PPAR α , β (previously referred to as δ), and γ , PPAR γ has been the most intensively investigated. This molecule participates in fundamental biological pathways of basic and clinical interest, such as cellular differentiation, insulin sensitivity, and carcinogenesis. Recent studies have demonstrated that PPAR γ is expressed in several carcinoma cells including lung carcinoma. Also, PPAR γ activation is involved in growth inhibition and differentiation of a variety of lung carcinoma cell types [131]. We have found that several commonly used drugs capable of stimulating PPAR γ can modulate the expression of fibronectin as well as COX-2 related genes in lung carcinoma cells [132]. PPAR γ ligands also inhibited $\alpha 5$ integrin expression in NSCLC through Erk-related signals [67]. *In vivo* studies in rats demonstrate that oral administration of the PPAR γ ligand thiazolidinediones (TZD) reduced extracellular matrix deposition in human colon cancer cells [133]. Also, TZD-induced PPAR γ activation inhibits fibronectin synthesis induced by transforming growth factor- $\beta 1$ [134]. These observations suggest a novel role for PPAR γ ligands in the development of therapeutic interventions able to prevent or inhibit lung carcinoma progression. Of course, not all PPARs are expected to be involved in lung carcinogenesis in the same fashion. A striking example of this is PPAR β/δ , which was recently shown to promote NSCLC proliferation [135].

CONCLUSION

Although much evidence implicates fibronectin in the pathogenesis of lung carcinoma growth, progression, and resistance to therapy, its role in lung carcinogenesis (and that of its integrin receptors) remains unclear. However, it appears that fibronectin, through multiple signaling pathways, stimulates tumor cell proliferation and protects against apoptosis. The relevance of this to lung cancer in humans needs further exploration in view of studies showing seemingly paradoxical findings. Nevertheless, it is possible that, depending on tumor stage, targeted inhibition of fibronectin and/or integrin-mediated cell survival signals may be of therapeutic benefit, leaving the cell vulnerable to chemotherapeutic attack. Also, it is possible that some of the anti-cancer effects of agents currently being used in the treatment of lung cancer may be related to effects on fibronectin expression or the generation of integrin-dependent signals. Clearly, interactions between lung carcinoma cells and their surrounding stroma represent a promising target in our quest against lung cancer. Therefore, studies directed at developing strategies that exploit this area are likely to accelerate our efforts against this devastating disease.

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