

Metastasis suppression by adiponectin

LKB1 rises up to the challenge

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Adiponectin is an adipocytokine involved in the pathogenesis of various obesity-related disorders. Also, it has been shown that adiponectin has therapeutic potential for metabolic syndrome, systemic insulin resistance, cardiovascular disease and more recently carcinogenesis. Adiponectin can modulate breast cancer cell growth and proliferation. Anti-metastatic effects of adiponectin have also been elucidated. It has been shown that adiponectin inhibits important metastatic properties such as adhesion, invasion and migration of breast cancer cells. Examination of the underlying molecular mechanisms has shown that adiponectin treatment increases AMP-activated protein kinase (AMPK) phosphorylation and activity. Adiponectin also increases phosphorylation of downstream target of AMPK, Acetyl-CoA Carboxylase (ACC) and decreases phosphorylation of p70S6 kinase (S6K). Importantly, adiponectin treatment increases the expression of tumor suppressor gene, LKB1 in breast cancer cells. LKB1 is required for adiponectin-mediated modulation of AMPK-S6K axis and more importantly, its biological functions including inhibition of adhesion, migration and invasion of breast cancer cells. Although further studies are required to analyze the effect of adiponectin on LKB1-AMPK-S6K axis, these data present a novel mechanism involving specific upregulation of tumor suppressor gene LKB1 by which adiponectin inhibits adhesion, invasion and migration of breast cancer cells. These results highlight a new role for

LKB1 in adiponectin action and may have significant implication for development of novel therapeutic options.

Cancer research has largely focused on the molecular basis of oncogenic transformation and tumorigenesis for many years. Recent progress in cancer research has put the metastatic process at the center stage because higher metastatic potential of tumor cells is the major cause of mortality from solid tumors. Metastasis is a complex process that involves modulation of various molecular signaling networks. Tumor cells alter the microenvironment, attain greater cellular adhesion along with better ability to invade and migrate to gain access to circulation. These wandering tumor cells defy anoikis, survive in the circulation, exit into new permissive organ site and colonize distant organs.¹ The microenvironment in which the tumor originates plays an important role in tumor initiation, progression and metastasis.

Adipocytes Modulate Breast Tumor Growth and Metastasis

With epithelial and other cells accounting for only approximately 10% of human breast volume, adipocytes make up the bulk of the human breast. In many human breast cancers, there is reduced connective tissue separating the adipocytes from tumor cells. Also, carcinomas invade through the basement membrane and infiltrate fibrous tissue barriers, resulting in close positioning that allows more paracrine interactions between the adipocytes and breast epithelial cells which might

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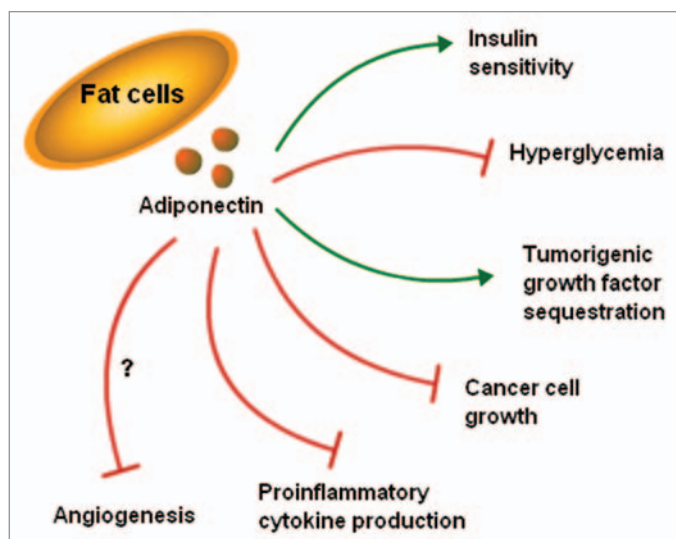


Figure 1. Adiponectin modulates the pathogenesis of various obesity-related disorders.

directly affect breast tumorigenesis.² In fact, paracrine effects of adipocytes on tumor cells have been demonstrated in various *in vitro* and *in vivo* pharmacological studies. Co-culture of mature rat adipocytes with breast carcinoma cells in three dimensional collagen matrix promotes proliferation of breast carcinoma cells.³ Treatment of MCF7 cells with conditioned media from murine adipocytes reveals upregulation of genes involved in invasion, proliferation and metastasis and downregulation of p18, a cell cycle checkpoint inhibitor and BARD1, a tumor suppressor protein in microarray analysis.⁴ Importantly, development of mammary tumors and metastasis is observed in mice injected s.c or i.p with the murine mammary carcinoma cell line.

SP1 and adipose tissue, while no tumor growth or metastasis is observed when SP1 cells are injected distant from any fat pad.⁵ In a direct comparison, 3T3-L1 murine adipocyte-secreted factors significantly stimulate MCF7 cell migration and proliferation when compared to fibroblast-secreted factors.⁶ The close relationship between adipocytes and breast cancer has also been shown by the finding that co-injection of SUM-159PT human breast adenocarcinoma cells and murine adipocytes results in the formation of three-times larger tumors in comparison to the smaller tumors resulting with co-injection of SUM-159PT cells with murine fibroblasts.⁴ Adipocytes have been considered

as merely cargo spaces for fat storage, to be released during times of hardship, such as fasting or starvation. More recently, adipose tissue has been recognized as an important endocrine organ as adipocytes can produce various hormones, cytokines and growth factors, collectively called adipocytokines.⁷ Many adipocytokines such as leptin, interleukin 6 (IL6) and tumor necrosis factor alpha (TNF α) have been shown to increase breast cancer cell proliferation and tumorigenesis.^{2,8-11} Based on these studies, it is widely believed that adipocytokines can play key roles in mediating the stromal-breast epithelial crosstalk and in influencing the growth and proliferation of tumor stroma and breast tumor cells.

Adiponectin Functions as a Protective Adipocytokine

Adiponectin¹²⁻¹⁵ is an important adipocytokine that has been known as a “guardian angel adipocytokine” owing to its protective role against the pathogenesis of various obesity-related disorders (Fig. 1) and the metabolic syndrome, particularly type 2 diabetes and cardiovascular disease.¹⁶⁻¹⁸ Therapeutic potential of adiponectin has been shown in various studies where replenishment of adiponectin in animal models reduces body weight, improves glucose/lipid homeostasis, increases insulin sensitivity, prevents atherosclerosis and fatty liver diseases.¹⁷ Adiponectin

suppresses proliferation and activation of immune cells,¹⁹ downregulates vascular adhesion molecules in endothelial cells and inhibits smooth muscle migration.²⁰ Adiponectin controls the bioavailability of certain growth factors by directly binding them hence preventing their interactions with respective receptors.²¹ More recent epidemiological, biochemical and molecular biological studies have established an important role for adiponectin in cancer.²² Adiponectin mediates its cellular functions in a tissue-dependent manner via its membrane bound receptors; AdipoR1 and AdipoR2. Recently, T-cadherin has been identified as an adiponectin receptor.²³⁻²⁵ Low levels of plasma adiponectin associated with obesity have been linked with many common forms of cancer in various epidemiological studies.^{22,26} Low serum adiponectin levels has been linked with higher risk of breast cancer in both postmenopausal and premenopausal women, independent of age, menopause status, hormone receptor status, lymph node metastasis, status of ER and Her2/neu.^{27,28} Breast tumors arising in women with low-serum adiponectin levels may have a more aggressive phenotype (large size of tumor, high histological grade and increased metastasis).²⁷⁻²⁹ Adiponectin exerts an anti-proliferative response in human breast cancer cells.³⁰⁻³⁵ Expression of adiponectin in breast tumor samples and adjacent tissues suggests that adiponectin secretion from mammary adipocytes might affect the malignant properties of breast cancer cells in the breast tumor microenvironment via paracrine interactions.³⁶

Molecular Mechanisms Underlying Adiponectin Function

Recent studies have shown the negative effect of adiponectin on the malignant properties of breast cancer cells. Taliaferro-Smith et al. show that adiponectin treatment inhibits malignant properties such as adhesion, invasion and migration of breast cancer cells.³⁷ How does adiponectin produce this profound change in metastatic properties of breast cancer cells? Investigating the underlying molecular mechanisms, they show that adiponectin stimulates AMPK phosphorylation and activity while reducing mTOR

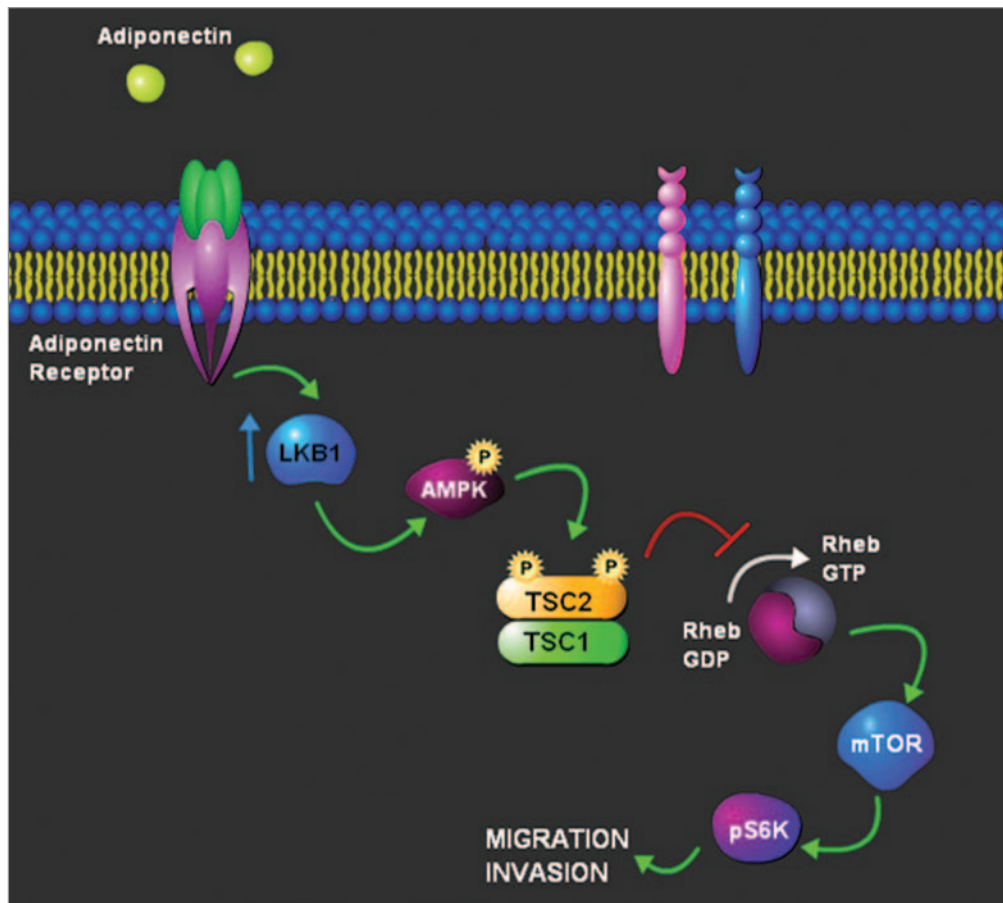


Figure 2. Model describing the underlying molecular mechanism involved in adiponectin mediated inhibition of migration and invasion of breast cancer cells. Adiponectin increases the expression of LKB1 resulting in increased phosphorylation of AMPK. AMPK activation results in modulation of TSC2/TSC1 leading to inhibition of S6K activation.

activity. Adiponectin reduces phosphorylation of S6K, a known target of mTOR. A very important aspect of this study is that adiponectin treatment increases the expression of tumor suppressor LKB1 (Fig. 2).³⁷ More importantly, LKB1 is not only required for adiponectin-mediated modulation of AMPK-S6K axis but also for adiponectin-mediated inhibition of adhesion, invasion and migration of breast cancer cells. This conclusion is supported by the findings that treatment of stable LKB1-null-MCF7 cells with adiponectin shows no adiponectin-mediated increase in AMPK phosphorylation or reduction in pS6K phosphorylation while LKB1-expressing MCF7 cells exhibit adiponectin-mediated increase in AMPK phosphorylation and decrease in pS6K phosphorylation. In addition, it is shown that adiponectin treatment does not affect migration and invasion potential of stable LKB1 nullMCF7. Hence,

adiponectin treatment significantly inhibits malignant properties of breast cancer cells via modulation of LKB1-AMPK-S6K axis.

Taliaferro-Smith et al. demonstrate for the first time that adiponectin can increase LKB1 expression in human breast cancer cells, which play a critical role in mediating the inhibitory effects of adiponectin on mammary tumorigenesis.³⁷ LKB1 kinase, a key determinant in Peutz-Jeghers syndrome, is known as a tumor suppressor gene that has an inherited propensity to gastrointestinal and other cancers including lung, pancreatic and breast cancer.^{38,39} Inactivation of LKB1 gene has been shown in a subset of sporadic lung and pancreatic cancer. A recent clinical study reporting extensive analysis of LKB1 expression in breast tumors using IHC show that while abrogation of LKB1 expression is not common in human breast carcinoma, it importantly

correlates with high-grade DCIS and high-grade invasive ductal carcinoma.⁴⁰ It is important to note that inhibition of LKB1 expression is only observed in the DCIS associated with invasion and not pure DCIS cases demonstrating that loss of LKB1 can potentially promote invasion. In agreement with these results, low LKB1 protein levels have been shown to correlate with poor prognosis in breast carcinoma.⁴¹ It has also been shown that adiponectin mediated increase in LKB1 expression diminishes invasion potential of breast cancer cells.³⁷ LKB1 participates in regulating various cellular functions including controlling cell polarity, energy homeostasis, protein synthesis and cell cycle arrest. Studies using mutant LKB1 lacking the nuclear localization signal have reported that cytoplasmic pool of LKB1 plays an important role in mediating its tumor suppressor properties. Co-expression of LKB1 with STRAD and MO25 strikingly

increase its localization in cytoplasm whereas mutant LKB1, unable to interact with STRAD and MO25 does not translocate to cytoplasm.⁴²⁻⁴⁴ It is speculated that adiponectin might also increase LKB1-STRAD-MO25 interaction in addition to overexpression of LKB1 thus increasing the functional pool of LKB1. Upstream kinase LKB1 itself gets phosphorylated at 8 residues (Ser31, Ser325, Thr366, Ser431 by upstream kinases and autophosphorylation at Thr185, Thr189, Thr336 and Ser404). The importance of these phosphorylation events is still questionable for LKB1 function as mutation of any of these sites of phosphorylation to either Ala to abolish phosphorylation or to Glu to mimic phosphorylation has thus far not been reported⁴⁵⁻⁴⁷ significantly affect LKB1 catalytic activity in vitro or its cellular localization.⁴³ Recent findings support the concept that LKB1 plays an integral role in breast cancer metastasis and higher expression of LKB1 in response to adiponectin treatment inhibits invasion and migration properties of breast cancer cells.

LKB1 Modulates AMPK-mTOR Axis

Tumor suppressor function of LKB1 can be partly due to its ability to activate master metabolic regulator AMPK.³⁹ Direct binding of AMP to the nucleotide-binding domain of AMPK induces a conformational change, exposing the activation loop of the catalytic kinase subunit. Upstream kinase LKB1 phosphorylates a critical threonine in this activation loop to activate AMPK.³⁹ AMPK activation is dependent on LKB1 as genetic depletion of LKB1 in mouse embryonic fibroblasts (MEFs) results in a loss of AMPK activation even in the presence of energy stresses that raise AMP.⁴⁸ Concurring to these findings, Taliaferro-Smith et al. show that silencing of LKB1 in breast cancer cells results in lower AMPK activation while overexpression of LKB1 increases AMPK activation which synergistically increases by concomitant adiponectin treatment.³⁷ AMPK is an important downstream target of LKB1, which upon activation, regulates the activation of two other tumor suppressors, TSC1 and

TSC2. Heterodimeric binding partners TSC2 and TSC1 regulate Rheb which is a critical regulator of mTOR protein kinase which in turn regulates a vast range of cellular activities, including transcription, translation, cell size, mRNA turnover, protein stability, ribosomal biogenesis and cytoskeletal organization.⁴⁸ Analysis of these molecular cascades suggest that cells having reduced expression of LKB1 show hyperactivation of mTOR that may contribute to carcinogenesis.^{39,48} Indeed, it has been observed that LKB1 silencing results in higher mTOR activity whereas adiponectin-induced higher expression of LKB1 results in inhibition of mTOR activity in breast cancer cells. Based on these observations, it is hypothesized that development of adiponectin analogues may prove to be an excellent therapeutic option for carcinomas characterized by hyperactive mTOR signaling such as human familial cancer syndromes all of which are characterized by hamartoma-type tumors.

Many epidemiological studies have shown that higher serum levels of most of the adipocytokines are associated with poor prognosis for obesity-related diseases including carcinogenesis. In contrast, adiponectin has shown promising insulin-sensitizing, anti-inflammatory and antiatherogenic activities both in vitro and in vivo studies. Serum adiponectin levels are dramatically decreased in obesity and various obesity-related diseases. In vivo studies using animal models have shown that treatment with adiponectin can improve glucose/lipid homeostasis, increase insulin sensitivity and prevent atherosclerosis hence suggesting clinical relevance of adiponectin treatment.⁴⁹⁻⁵² Recent studies show that adiponectin inhibits the metastatic properties of breast cancer cells via upregulation of LKB1 and modulation of LKB1/AMPK-S6K axis thus providing mechanistic insights for the development of adiponectin analogues. Future studies may focus on the detailed mechanism of action of LKB1, the signaling pathways it uses and the possible interaction with other adipokines that might act in synergy. Is adiponectin the crucial, long-sought link between obesity and breast cancer? Although at this point adiponectin is unlikely to fully explain the relationship, there may

be more interactions with other signaling pathways as well as other adipokines. These findings are important enough to lend credence to the idea that adiponectin or LKB1, its crucial downstream target, might represent exciting targets for the drug development. Thus, increasing adiponectin levels using adiponectin analogues or augmentation of its effectiveness by using TZDs Thiazolidinediones, can potentially become a future beneficial treatment for breast cancer patients for inhibiting invasion and migration properties of breast tumors and improving overall survival.

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