Molecular Pathways: Adiponectin and Leptin Signaling in Cancer

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Abstract
The increasing percentage of obese individuals in the population and its independent association of increased risk for the development of cancer have heightened the necessity to understand the molecular mechanisms that underlie this connection. The deregulation of adipokines in the setting of obesity and their impact on cancer progression and metastasis is one such area of research. Adipokines are bioactive proteins that mediate metabolism, inflammation, angiogenesis, and proliferation. Altered levels of adipokines or their cognate receptors in cancers can ultimately lead to an imbalance in downstream molecular pathways. Discovery of adipokine receptors in various cancers has highlighted the potential for novel therapeutic targets. Leptin and adiponectin represent two adipokines that elicit generally opposing molecular effects. Epidemiologic studies have highlighted associations between increased serum leptin levels and increased tumor growth, whereas adiponectin exhibits an inverse correlation with cancer development. This review addresses the current level of understanding of molecular pathways activated by adiponectin and leptin to identify the areas of intervention and facilitate advancement in the field. Clin Cancer Res; 19(8); 1926–32.
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Background
A strong correlation between obesity and cancer, coupled with the rising obesity epidemic, has led to a prediction of an increase in forthcoming new cancer cases. Obesity commonly leads to deregulation of adipokines, bioactive proteins primarily secreted from adipocytes, which elicit their biologic effects upon binding to cognate receptors. The primary role of adipokines is to help maintain metabolic homeostasis, yet expanded roles for adipokines have shown their ability to modulate inflammation, angiogenesis, proliferation, and apoptosis. With these processes in mind, a role for adipokines in cancer progression and metastasis has become apparent. The majority of cancer-related studies have focused in vitro on the ability of adipokines to affect the typical hallmarks of cancer, including proliferation, evasion of apoptosis, tumor cell migration and invasion, angiogenesis and vascular stimulation, and evasion of immune detection. More pertinent are preclinical studies that have validated the impact of adipokines on cancer progression in vivo, yet the signaling mechanisms through which these adipokines are mediating oncogenic phenotypes still require further elucidation. This review will address the molecular pathways of 2 prominent adipokines, leptin and adiponectin, and the potential to develop novel cancer therapeutics.

Adipokines: leptin and adiponectin
Leptin is a 16 kDa bioactive protein encoded by the Ob gene, secreted from adipocytes as well as other tissues, which acts as a regulator of energy to control satiety through stimulation in the central nervous system as well as to modulate glucose and insulin homeostasis through activation in peripheral tissues (1). Leptin typically circulates in the blood at a concentration of 5 to 10 ng/mL in healthy patients, yet its level increases in obese and diabetic patients upward of 50 ng/mL (2). Leptin stimulates a specific set of receptors from the extended class I cytokine receptor family, comprising 6 isoforms that dimerize with each other, but lacks intrinsic kinase activity (3). Leptin receptor isoforms vary with respect to tissue and cell type as well as with respect to ligand stimulation. Autoregulation of receptor levels as well as ligand-dependent activity may additionally lead to leptin resistance (4, 5).

Adiponectin is a part of the complement-1q family of proteins that is primarily secreted by adipocytes as a monomeric protein, which can further oligomerize to form low-molecular weight, high-molecular weight (HMW), and multimeric complexes (6). In addition, adiponectin can be cleaved by leukocyte elastase to generate a globular oligomeric complex (7). Adiponectin is generally maintained between 7 to 15 µg/mL in the plasma of healthy humans...
and exhibits a negative correlation with body mass index as well as the percentage of body fat (8, 9). Adiponectin activates 2 main seven-transmembrane receptors, adiponectin receptor 1 (adipoR1) and adiponectin receptor 2 (adipoR2; ref. 10). AdipoR1 has a greater affinity for globular adiponectin, whereas adipoR2 binds full-length and multimeric adiponectin more avidly (10). Stimulation of either receptor leads to regulation of metabolic effects through the activation and phosphorylation of AMPK, acetyl-CoA carboxylase (ACC), as well as p38 mitogen-activated protein kinase (MAPK; ref. 10). Knockout of each receptor resulted in an opposition of effects on locomotor activity and metabolism, where adipoR1 was shown to be associated with increased adiposity and decreased glucose tolerance while adipoR2 is resistant to diet-induced obesity (11, 12).

Antagonistic signaling between leptin and adiponectin

Leptin and adiponectin generally affect cellular behavior in an opposing manner. Highlights of these studies suggest that adiponectin administration in vivo has been shown to decrease growth and proliferation, increase apoptosis, decrease invasion, and decrease vessel density in murine cancer models (13–17). Leptin has been shown to increase proliferation, migration, and invasion of cancer cells (18–25) as well as contribute to release of VEGF (26). The ratio of leptin to adiponectin was recently described to be a potential key factor for outcome when assessing plasma levels (27). An important aspect of this consideration is that adiponectin can antagonize the actions of leptin. The molecular mechanisms through which adiponectin and leptin affect cancer cell behavior still require further elucidation. Figure 1 illustrates the dynamic signaling pathways for leptin and adiponectin, which we have combined to ascertain common mediators as potential key components for therapeutic intervention.

Leptin binding to all 4 forms of the short leptin receptor (Ob-Ra, Ob-Rc, Ob-Rd, and Ob-Rf) elicits activation of Janus-activated kinase (JAK)2 and subsequent phosphorylation of insulin receptor substrates (IRS), initiating activation of the phosphoinositide 3-kinase (PI3K)/Akt pathway (3). The long form of the receptor (Ob-Rb) contains an intracellular carboxy terminal extension that provides an additional 3 tyrosine residues (Tyr985, Tyr1077, and Tyr1138), necessary to confer binding and activation of STAT3 and STAT5 (3, 28, 29). In addition, a secreted isoform lacking the intracellular signaling domains (Ob-Rc; ref. 30) functions to sequester and block leptin-induced STAT3 activation (31).

Leptin-dependent activation of JAK2 additionally confers phosphorylation of both Tyr985 and Tyr1138 as well as activation of IRS1/2. Phosphorylation of Tyr985 is essential for phosphorylation of Tyr1138, which promotes Src-mediated activation of STAT3 (28). In addition, phosphorylation of Tyr985 promotes recruitment of SH2P2, a protein phosphatase, and SOCS3, an inhibitor of STAT3 (32). Leptin-mediated SH2P2 binding leads to activation of extracellular signal-regulated kinase (ERK; refs. 33, 34) as well as attenuation of p62Dok (35), a RasGTPase, leading to activation of Ras and subsequent proliferation. SOCS3 and PPAR-γ are upregulated via activation of STAT5 at Tyr1077 subsequent to leptin stimulation (32). Leptin-mediated upregulation of SOCS3 is thought to be involved during chronic leptin stimulation, which then acts as a negative regulator to directly bind and block Ob-Rb signaling as well as JAK2 activity (36, 37). In addition, adiponectin can increase protein tyrosine phosphatase 1B (PTP1B), which then dephosphorylates STAT3 as well as JAK2, further antagonizing leptin signaling (38).

Adiponectin binding can occur through either receptor 1 (AdipoR1) or receptor 2 (AdipoR2), which homodimerize or heterodimerize (6). While globular adiponectin (gAdN) preferentially binds to adipoR1, HMW adiponectin preferentially stimulates adipoR2 (10). Knockout studies suggest that adipoR1 is necessary for AMPK activity, whereas adipoR2 is necessary for PPAR-α activity, yet both receptors have been shown to be able to increase phosphorylation of AMPK (11, 12). APPL1, a pleckstrin homology adaptor protein, binds to the intracellular portion of the adiponectin receptors and participates in AMPK activation leading to GLUT4 membrane translocation, p38 MAPK activation, and phosphorylation of ACC (39). AMPK activation inhibits the mTOR complex via Raptor in the mTorc1 complex as well as activating TSC2, an inhibitor of mTOR (40). Phosphorylation of AMPK further activates TP53 (41) and proapoptotic pathways as well as the activation of protein phosphatase 2A (PP2A), which can negatively regulate Akt in response to adiponectin stimulation (42) and therefore antagonize leptin-induced Akt. Adiponectin stimulation of APPL1 alternately activates Akt to enhance mTOR in the absence of PTEN (43), which normally inhibits phosphoinositide-3-kinase (PI3K) activation of Akt. Therefore, cross-talk between leptin and adiponectin as well as the activation of multiple pathways keep proliferative signaling in balance.

Leptin and Leptin Receptors in Cancer

Tumor-associated leptin receptor levels are thought to contribute to tumor growth and progression. Increased detection of ObR in ovarian cancers was correlated with decreased survival (44). Leptin receptor expression is enhanced in 83% of human breast cancers, and 34% of patients with high leptin receptor level and high ligand level had detectable distant metastases (45). In the murine MMIV-TGF-α model, deficiency in the long form of the leptin receptor (Db/Db) resulted in failure of mammary tumor formation (46). Knockdown of the ObR through siRNA in MCF-7 breast cancer cells resulted in suppression of tumor volume in a mouse xenograft model (47). Knockdown of the long form of the leptin receptor can abolish integrin-dependent migration of chondrosarcoma cells through involvement of IRS-1/PI3K–dependent activation of Akt (48). In addition, pancreatic tumors grown in leptin receptor–mutant mice (Lept−/−) had larger tumors and more metastases when compared with wild-type mice (49). In addition, mutational status may affect receptor function.
Three single-nucleotide polymorphisms in the leptin receptor gene (K109R, K656N, and Q223R) showed an association with increased basal-like breast cancer risk (50). These results suggest that tumor leptin receptor levels directly influence growth and progression.

Circulating levels of leptin have been investigated to determine the correlation with cancer and progressive disease. Elevated leptin levels in patients with cancer compared with normal or preoperative levels have been reported in hepatocellular carcinoma and prostate cancer, whereas levels are relatively unchanged in patients with breast cancer (51–55). However, in patients with pancreatic and colon cancer, leptin levels were generally found to be decreased (56–59). Complications such as pancreatic dysfunction, advanced progression of disease, weight loss, and/or cachexia might be underlying factors for decreased leptin levels. Leptin produced by adjacent adipose might provide a local increased level of stimulation to tumors (60–62), suggesting that the presence of tumor-associated adipose represents an important microenvironmental influence. Although normally secreted from adipose, autocrine mechanisms for leptin are an important consideration since leptin can be secreted from glioblastoma and breast cancer cells (63, 64). Furthermore, intratumoral mRNA leptin levels in patients with high leptin receptor levels correlated with decreased relapse-free survival (55).

Adiponectin and Adiponectin Receptors in Cancer

Epidemiologic studies show that low levels of adiponectin have an inverse association with risk for the
development of multiple cancers as well as advanced progression of disease (65). Two adiponectin single-nucleotide polymorphisms have been shown to increase prostate, colon, and breast cancer risk (66–68). Adiponectin deficiency through the use of knockout mice has shown accelerated hepatic tumor formation (69) and increased colon polyp formation (70), yet it delayed tumor growth in a mammary MMTV-PyV-mT model due to decreased vascularization and increased apoptosis in early stages of the disease (71, 72). Tumor-promoting effects are likely secondary to initiation, but no clear studies have implicated adiponectin as an initiator of cancer development.

The adiponectin receptors have been detected in gastric, colon, prostate, breast, pancreatic, and many other cancers (16, 56, 73–75). Detection of adiponectin receptors in gastric cancers was associated with longer overall survival (76). Two single-nucleotide polymorphisms of adipor1 associate with prostate cancer risk and one with breast cancer risk (67, 68). Six genetic associations in the adipor1 and adipor2 genes have been detected in diabetic patients (77). Deletion of the adipor1, but not adipor2, resulted in a promotion of epithelial cell proliferation and increased number of aberrant crypt foci in a murine model (70). Future studies addressing the functional role of each adipor in cancer initiation and progression will add a substantial contribution to our understanding of the importance of adiponectin signaling in these diseases.

Clinical–Translational Advances

Preclinical advances

Currently, preclinical advances modulating adipokines have been limited for cancer therapeutics. Recombinant leptin treatment increased MDA-MB-231 breast tumor xenograft growth (22) as well as melanoma (78). In an animal study, female mice in the MMTV-TGF-α breast cancer model failed to develop tumors when crossed with leptin-deficient mice (46). Conversely, leptin antagonist treatment was shown to decrease the growth of triple-negative breast tumors in mice (79) as well as decrease 4T1 mouse mammary tumor growth in vivo through reduced VEGF, pSTAT3, and cyclin D1 (80). Recent evidence suggests that C-reactive protein as well as soluble leptin receptor can act to bind circulating leptin and attenuate its activity (81, 82). This provides insight into novel mediators of leptin action that may mediate its activity in patients with cancer. Antileptin therapy could potentially be used to decrease circulating levels of leptin or to alter the adiponectin:leptin ratio in patients with cancer, although additional preclinical studies will be needed to test the impact of altered leptin and adiponectin signaling in vivo.

Adiponectin treatment decreases the number of polyps, especially those larger in size, in the ApcMin intestinal tumor model (83). Adiponectin treatment induced apoptosis of gastric cancer cells in vitro, whereas in vivo its infusion into mice led to decreased metastasis (16). In addition, liver tumor growth and lung metastases were lowered by adiponectin overexpression (14). Interestingly, rosiglitazone treatment increased adiponectin serum concentrations (84) as well as adipor expression (85). In addition, hypocaloric diet and exercise led to an altered oligomeric distribution of adiponectin as well as increased adipor1 and adipor2 expression (86).

Clinical advances

The administration of leptin, adiponectin, or direct antagonists of either of these adipokines has not been reported in the literature for the treatment of human cancers. Leptin therapy was shown ineffective for patients with type II diabetes, yet it did improve insulin sensitivity in leptin-deficient patients (87). Currently, clinical applications of adiponectin and leptin therapeutics are more likely to address metabolic disorders, obesity, and diabetes than cancer therapeutics. However, the application of antileptin therapy or administration of adiponectin could both provide straightforward treatment options in cancer therapeutics through direct interactions in cancer cells or indirectly by reducing obesity and metabolic disorders, which have been associated with increased risk for cancer.

Alternatively, targeting downstream adipokine signaling mediators is likely to be an advantageous choice. Downstream targeting of the adiponectin with metformin can lead to the activation of AMPK. Metformin is gaining wide attention for its role as an antiobesogenic as well as its anti-tumor effects for breast, prostate, lung, colon, and ovarian cancers (88). Metformin therapy preceding cancer diagnosis was associated with better survival in diabetics as well as nondiabetics (89). Metformin and thiazolidinedione use among a defined patient population or diabetics with either stage 2 to advanced HER2 breast cancer or those with prostate cancer associated with decreased mortality (90, 91). Thiazolidinediones, which are PPAR-γ agonists and include pioglitazone and rosiglitazone, increase the secretion of HMW adiponectin from adipocytes (92). Recent data from randomized controlled trials indicated that thiazolidinedione use provides a modest decrease in the risk for lung, colorectal, and breast cancers (93). In addition, administration of a cholesterol-reducing drug, fenofibrate, increased plasma adiponectin concentration (94). Mechanisms to target the leptin pathway include the use of common pathway inhibitors such as STAT3 inhibitors (95). Akt inhibitors (96), and RAF inhibitors (97). Novel mechanisms of adipokine modulation through PTP1B and PP2A may additionally be used to inhibit the leptin receptor. Dual targeted therapies directed toward decreasing response from leptin stimulation and increasing the response from adiponectin pathways have the potential for more efficacious cancer therapy.

Conclusions

Obesity is a growing clinical problem and is independently associated with multiple cancers (98). This review illustrates that adipokines contribute to multiple aspects of cancer progression and elicit a broad range of effects in normal as well as transformed cells. Adipokine stimulation
seems not to follow a straightforward direct pathway, but instead contributes to a highly integrated cellular response. Determining circulating levels of adipokines as well as their receptors is equally important in determining which pathways are active and dominant. In addition, cancers acquire genetic mutations and epigenetic modifications that can result in activation of oncogenes such as Ras, RAF, ERK, and Akt or that can result in inactivation of tumor suppressors such as p53 and PTEN. In the future, we will likely have to consider individualized mutational status for cancer as well as in cancer cell lines to understand the impact these alterations have on adipokine signaling pathways. Integration of these aspects will then allow for targeted therapeutics and manipulation of adipokine pathways in cancer.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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