IS LEPTIN A PRO-OR ANTI-APOPTOTIC AGENT?

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ABSTRACT

Apoptosis, the regulated destruction of a cell, is characterized by biological and morphological changes and involves a large web of integrating pathways and factors. Apoptosis is necessary to eliminate excess cells and cells that hinder development, and hence the importance of apoptotic pathways and apoptotic agents in removing adipocytes for the treatment of obesity has been recently explored. Leptin was widely recognized for its ability to regulate adipose tissue mass by influencing food intake and energy expenditure. Recent findings, however, demonstrated that leptin treatment initiated apoptosis in adipose tissue. Leptin-induced adipocyte apoptosis was a surprising finding, as adipocytes were thought to be extremely stable; however, both pro- and anti-anti-apoptotic effects of leptin have been demonstrated in several cell types. In particular, anti-apoptotic effects have been shown in certain types of cancer cells, and are correlated with the presence of leptin receptors. While leptin's effects on energy balance, including induction of adipocyte apoptosis, are primarily mediated by the central nervous system, it is possible that anti-apoptotic effects of leptin are mediated through autocrine or paracrine effects. In this chapter both anti-apoptotic and pro-apoptotic effects of leptin in several cell types are reviewed.

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INTRODUCTION

Leptin is a cytokine-like hormone that is expressed primarily in adipose tissue, with low levels also detected in the skeletal muscle, placenta, and the brain (Trayhurn and Beattie, 2001). Leptin is a non-glycosylated protein with a molecular mass of 16 KD (Harvey, 2003). Its expression in adipose tissue is up-regulated by a variety of factors, such as glucocorticoids, acute infection, and pro-inflammatory cytokines, and is down-regulated by cold exposure, adrenergic stimulation, and growth hormone (Trayhurn and Beattie, 2001). Leptin was originally discovered as the missing protein in the genetically obese ob/ob mouse (Halaas et al., 1995), and it clearly plays a role in regulation of body fat content, in part through effects on eating behavior, body temperature, physical activity, lipid mobilization and adipose tissue apoptosis (Choi et al., 2005; Gullicksen et al., 1999; Qian et al., 1998a). However, it also is involved in the regulation of bone formation (Elefteriou et al., 2004; Gordeladze et al., 2002; Gullicksen et al., 2003). In addition, leptin has a concomitant fluctuation with seasonal changes and is probably involved in seasonal control of body fat (Bartness et al., 2002). Thus, leptin is a hormone with multiple functions in most of the body systems (Friedman, 2002).

Consistent with leptin having structural similarities to cytokines, the leptin receptor (ObR) belongs to the cytokine receptor class I superfamily (Tartaglia et al., 1995). Several isoforms of ObR (a, b, c, d, e) with different lengths of C-termini have been identified (Lee et al., 1996). The long form of the receptor, ObRb mediates most leptin functions and the short form, ObRa, facilitates transport of leptin via the blood- brain barrier (Tartaglia, 1997). Leptin receptors are widely expressed throughout the brain in areas such as the cortex, cerebellum, brainstem, basal ganglia, and hippocampus (Harvey, 2003), and it is generally accepted that the hypothalamus is the critical action site for leptin's effects on energy balance (Elmquist, 2000; White et al., 2000). Further, the presence of leptin receptor mRNA in the meninges and the microcirculation implies that leptin receptors at one or all of these sites are responsible for transporting leptin into or out of the CNS (Elmquist et al., 1998).

The finding of structural similarity between leptin and its receptor and cytokine-receptor systems that control hematopoiesis prompted investigations showing that leptin stimulated blood cell formation and proliferation (Gainsford et al., 1996). In contrast, Prins et al. suggested that leptin might decrease adipocyte volume and number via its CNS action, thereby influencing adipose tissue mass (Prins and O'Rahilly, 1997). Thus, contradictory findings on the effects of leptin and leptin receptors on cell proliferation and apoptosis have been reported. Before discussing the anti- and pro-apoptotic effects of leptin, we will briefly discuss the types of apoptotic pathways and molecular events leading to this process.

Apoptosis: a Basic Biologic Phenomenon

Apoptosis is considered the principal mechanism of "programmed cell death" in mammalian tissues. The term "apoptosis" was first devised in 1972 after identifying morphologically similar cell deaths in many pathological conditions as well as in normal tissue (Kerr et al., 1972). Apoptosis, also conceptualized as a self directed-cellular "suicide", is different from other types of cell death like necrosis or oncosis (Majno and Joris, 1995). However, other forms of programmed cell death, namely aponecrosis (Formigli et al., 2000), autophagy (Debnath et al., 2005) and paraptosis (Sperandio et al., 2004), have also been described

recently. The morphological features of apoptosis are similar across cell types and species (Abrams et al., 1993). Cell shrinkage, chromatin condensation, cellular budding and rapid phagocytosis by phagocytes or adjacent cells are the typical events of apoptosis that occur in fixed sequence. Light and electron microscopy have been used to identify the various morphological changes that occur during apoptosis (Hacker, 2000).

The rapid increase in the number of publications on apoptosis over the past two decades followed identification of several genes and molecular pathways that are involved in the execution of apoptosis. Apoptosis is a coordinated and energy-dependent process involving a cascade of molecular events. Two apoptotic pathways identified to date are the extrinsic, or death receptor pathway, and the intrinsic, or mitochondrial pathway. However, these two pathways are linked and molecules in one pathway influence the other (Igney and Krammer, 2002).

1. Extrinsic Pathway

This pathway is triggered by the binding of an extracellular ligand to a death receptor which belongs to tumor necrosis factor (TNF) receptor gene superfamily (Locksley et al., 2001). Death factors, Fas ligand (FasL) or tumor necrosis factor (TNF), bind to their death receptors and induce apoptosis, killing the cells within hours. Members of the TNF receptor family carry cysteine-rich extracellular domains and have a cytoplasmic domain of about 80 amino acids called the "death domain" (Ashkenazi and Dixit, 1998). FasL and Fas receptor interaction leads to binding of cytoplasmic adapter protein, FADD (Fas associated death domain) and TNFα/TNF receptor interaction leads to binding of TRADD (TNFR1-associated death domain) with recruitment of FADD (Hsu et al., 1995; Wajant, 2002). FADD subsequently binds to the prodomain of caspase-8 and a complex, termed death-inducing signaling complex (DISC), is formed which further activates caspase-8. Caspase-8 then activates a series of downstream caspases that result in cleavage of structural and regulatory intracellular proteins, ultimately leading to apoptosis (Chinnaiyan et al., 1996).

2. Intrinsic Pathway

The stimuli activating this pathway are not receptor mediated, but the signals produced act within the cell and lead to mitochondrial initiated events. Oligomerization of two pro-apoptotic proteins, Bax and Bak in the mitochondrial outer membrane activates this pathway resulting in an opening of the mitochondrial permeability transition (MPT) pore, loss of the mitochondrial transmembrane potential and release of cytochrome *c*, second mitochondria–derived activator of caspase (Smac/DIABLO), and Omi stress-regulated endoprotease/high temperature requirement protein A2 (Omi/HtrA2) and the serine protease HtrA2/Omi (Du et al., 2000; Garrido et al., 2006; Suzuki et al., 2001). These proteins activate the caspase-dependent mitochondrial pathway. The release of a second group of pro-apoptotic proteins, apoptosis inducing factor (AIF), and endonuclease G from the mitochondria is a late event that occurs after the cell has committed to die. AIF translocates to the nucleus and causes DNA fragmentation into approximately 50-300 kb pieces and condensation of peripheral nuclear chromatin (Joza et al., 2001).

The activation of the execution caspases, caspase-3, caspase-6, and caspase-7 begins the process of apoptosis. These caspases activate cytoplasmic endonucleases, and proteases that

degrade and cleave nuclear material and cytoskeletal proteins like cytokeratins, poly(ADP-ribose) polymerase (PARP), the nuclear matrix protein NuMA and others (Slee et al., 2001), resulting in morphological and biochemical changes seen in apoptotic cells.

Anti-apoptotic Effects of Leptin

Although the discovery of leptin was based on its ability to regulate adipose tissue mass, leptin was later considered a multifunctional factor in the development of hematopoietic, reproductive and immunologic functions (O'Brien S et al., 1999; Tessitore et al., 2004). Mitogenic effects of leptin on breast cancer cells (Dieudonne et al., 2002), colonic epithelium (Hardwick et al., 2001), gastric mucosal cells (Schneider et al., 2001) and osteoblastic cells (Gordeladze et al., 2002) have also been reported recently. Although most cases of obesity are characterized by an increase in leptin levels, leading to resistance to leptin's effects on body fat regulation (Frederich et al., 1995), some cell types may not become resistant to leptin. For example, it has been reported that cancer cells are responsive to leptin, thus the high circulating levels of leptin that occur with obesity may be associated with the increased incidence of cancer in obese individuals (Somasundar et al., 2003).

Leptin has been shown to activate estrogen receptor α (ERα) through mitogen activated protein kinase pathway (MAPK) in breast cancer cells (Catalano et al., 2004), and high levels of interleukin-1alpha (IL-1α) produced by breast cancer cells stimulated the expression of leptin (Kumar et al., 2003). Increased leptin further induced the transcription of aromatase, followed by activation of activator protein 1 (AP-1), signal transducers and activators of transcription 3 (STAT3) and extracellular signal regulated kinase 2 (ERK 2) which promoted estradiol synthesis (Catalano et al., 2003).(May be we can include a picture showing the effect of leptin on cell proliferation – see figure from a paper below). Confirming these findings, leptin abrogated anti-cancerous effects of estrogen antagonists in vitro leading to proliferation of breast cancer cells that are positive for ERα (Garofalo et al., 2004). Thus it is possible that elevated serum leptin levels might lead to the development of antiestrogen resistance in obese individuals suffering from breast cancer.

Recent studies show that leptin affects the risk of clinically relevant prostate cancer through testosterone, a well-established mitogen (Chang et al., 2001). Leptin also stimulated cell proliferation, migration and angiogenesis in prostate cancer cells by increasing the expression of vascular endothelial growth factor (VEGF), transforming growth factor (TGF-β1), and basic fibroblast growth factor (bFGF). In addition, leptin increased the migration of normal epithelial cells (Sierra-Honigmann et al., 1998). Therefore, high leptin levels associated with obesity may be a risk factor in prostate cancer patients (Frankenberry et al., 2004). Similarly leptin stimulated proliferation of insulin-secreting tumor cell lines (Okuya et al., 2001), although when pancreatic cells are treated with leptin in vitro, the mitogenic effects are not observed. In fact the growth of pancreatic cells was significantly reduced with leptin treatment (Somasundar et al., 2003). Leptin also serves as a survival signal in a variety of cell types, including T cells, macrophages and neutrophils, by enhancing proliferative and antiapoptotic activities. Some of the mechanisms involved in leptin's antiapoptotic effects in eosinophils include delayed cleavage of proapoptotic Bax proteins, mitochondrial release of cytochrome c, and second mitochondria-derived activator of caspase (Conus et al., 2005).

Leptin is believed to play an important role in regulating the onset of puberty (Friedmann & Halaas, 1998); e.g., treatment of female mice with leptin accelerated the maturation of the

reproductive tract leading to an early onset of the estrous cycle and reproductive capability (Chehab et al. 1997; Ahima et al . 1997). A surge in plasma leptin concentration observed in prepubertal males (humans) further supports the role of leptin in puberty (Mantzoros et al., 1997). Leptin replacement therapy has been successful in restoration of spermatogenesis and reproductive function (Mounzih et al., 1997) in leptin-deficient ob/ob mice. Since the identification of leptin receptors on germ cells and Leydig cells within the testis (Caprio et al., 2003; El-Hefnawy et al., 2000), a direct regulatory role for leptin in reproduction had been suggested, as the impaired spermatogenic process in the leptin-deficient mouse was due to elevated germ cell apoptosis (Bhat et al., 2006). Treatment with exogenous leptin had advanced the onset of attenuation of ovarian apoptosis and enhancement of folliculogenesis (Paczoska-Eliasiewicz et al., 2006) and maturity (Almog et al., 2001). As a balancing act of homeostasis in developing corpora lutea, leptin acts as a pro- or antiapoptotic factor, which reverses the antiapoptotic action of IGF-I in order to protect cells from excessive apoptosis in maintaining appropriate cell number (Gregoraszczuk and Ptak, 2005).

Leptin promotes cognitive function and neural survival during adverse conditions. During conditions of energy depletion, AMPK reacts to minimize neural insult and enhances neurogenesis, but in severe conditions, it redirects neuronal fate towards apoptosis (Dagon et al., 2005). Under such conditions of impaired learning and memory, restoration of leptin function inhibits AMPK activation, thereby improving cognitive function (Randt et al., 1971), inhibiting neural apoptosis and inducing neural survival (Dagon et al., 2005).

The differential effects of leptin on cancer cells are thought to be mediated by leptin receptors. A number of cancer cell lines were reported to express leptin receptor isoforms, including breast cancer, choriocarcinoma and colon carcinoma cells (Attoub et al., 2000; Dieudonne et al., 2002; O'Neil et al., 2001). However, the expression and involvement of intracellular mediators like STAT and JAK in the activation of leptin and expression of leptin receptor needs further investigation. The cytokine-inducible inhibitors of STAT signaling (Naka et al., 1997) like suppressors of cytokine signaling (SOCS) that limit cellular response to leptin (Wang and Campbell, 2002) might contribute to the differential effects of leptin on different types of cancer cells. Thus, circulating leptin might act as a growth factor for development of several cancers in vivo supporting the link between obesity and risk of cancer proliferation (Table 1).

Pro-apoptotic Effects of Leptin

Although leptin can regulate adiposity indirectly by influencing food intake and energy expenditure (Zhang et al., 1999), interest in leptin stems from its weight reducing effects in rodents (Halaas et al., 1995). Both central and peripheral administration of leptin resulted in a dose-dependent decrease in body weight in both ob/ob (genetically obese mice) and wild-type mice (Ahima and Flier, 2000; Halaas et al., 1995), and this reduction was greater in ob/ob mice than pair-fed mice (Pelleymounter et al., 1995). The weight reducing effect of leptin in rodents was later shown to be partly a result of induction of adipocyte apoptosis (Qian et al., 1998a). Extreme depletion of adipose depots and prolonged recovery time for repletion of fat stores following long-term leptin treatment suggests that leptin decreases adiposity not only by stimulating lipolysis but also by triggering apoptosis (Della-Fera et al., 2001; Gullicksen et al., 2003). In contrast, as discussed in the previous section, leptin acts as either an anti-apoptotic agent or a mitogen in several other cell lines.

Apoptosis has been studied extensively in cancer cells, but until recently apoptosis has not been considered a part of normal adipocyte life cycle. Adipocytes were considered an extremely stable cell type, and Prins et al. (Prins and O'Rahilly, 1997) first reported that fat mass is balanced by cell acquisition and deletion, and that apoptosis plays an important role in the regulation of adipose tissue mass in humans. This report triggered several studies to investigate adipocyte apoptosis, including reports that ICV (intracerebroventricular) leptin induced adipocyte – specific apoptosis in rats (Qian et al., 1998a). The mechanism, however, through which leptin induces apoptosis in adipocytes is not fully understood.

Studies indicate that the effective dose of leptin when administered centrally is much lower than effective peripheral doses (Satoh et al., 1999). In addition, we have shown that leptin does not act directly on adipocytes to cause apoptosis (Ambati et al. 2007). So it is possible that leptin's effects on adipocyte apoptosis are mediated via increased sympathetic activity. Moreover, the beta-2 adrenergic agonist, clenbuterol, increased adipose tissue apoptosis in vivo (Page et al., 2004), which further supports the hypothesis that leptin might be acting through the CNS to induce adipocyte apoptosis via adrenergic stimulation. Adrenergic stimulation activated uncoupling protein 1 (UCP-1), which uncouples mitochondrial respiration in brown adipose tissue (BAT) (Nicholls and Locke, 1984) and white adipose tissue (WAT) (Yoshitomi et al., 1998). Interestingly, mRNA levels of uncoupling protein 2 (UCP-2), which is present in white adipose tissue and not BAT, increased more than 10-fold upon leptin treatment. This report supports the possibility that UCP-2 might be playing an important role in leptin induced adipocyte apoptosis (Fleury et al., 1997). Uncoupling of mitochondrial respiration in adipose tissue promotes free radical generation, like reactive oxygen species (ROS), which are directly involved with the induction of apoptosis (Negre-Salvayre et al., 1997). Leptin has also been found to increase ROS in endothelial cells by increasing fatty acid oxidation via protein kinase A activation (Yamagishi et al., 2001), and by stimulating polymorphonuclear neutrophils in response to infection (Caldefie-Chezet et al., 2001).

Leptin has also been shown to induce the expression of angiopoietin (Ang-2) in adipose tissue, which coincided with initiation of apoptosis in adipose endothelial cells leading to adipose tissue regression (Cohen et al. 2001). Likewise, leptin administration also decreased Bcl-2̃Bax ratio, while an increase in the Bcl-2̃Bax ratio in the fat pads during recovery is seen to prevent cell loss as the tissues show a tendency to return to control levels Leptin has also been reported to induce β-cell apoptosis by enhanced release of IL-β and suppressed release of IL-1 receptor antagonist in human pancreatic islets (Maedler et al., 2004), thus contributing to the loss of β-cells in diabetes (Donath et al., 2005).

While we cannot assume leptin-induced adipose apoptosis to be mediated only centrally, when tested in *in vitro* experiments, leptin did not act directly to induce adipocyte apoptosis (Ambati et al., 2007), thus supporting the hypothesis that leptin acts primarily centrally by initiating neural or humoral signals to induce adipocyte apoptosis. In addition, peroxisome proliferator-activated receptor γ (PPARγ), which activates adipocyte differentiation through transactivation of adipocyte specific genes, also triggers both apoptotic and anti-apoptotic pathways (Chawla and Lazar, 1994), and leptin caused a 70–80% increase in PPARγ expression in the epididymal fat pad (Qian et al., 1998b). Apart from regulating feeding behavior, leptin might also control intestinal function through regulation of apoptosis in jejunal and ileal mucosa (Lin et al., 2005).

CONCLUSIONS

Leptin promotes cell proliferative and has anti-apoptotic effects in a variety of cancer and normal cell types. Supraphysiological levels of leptin can cause dramatic reductions in body weight and fat in laboratory rodents, and leptin-induced adipose tissue apoptosis could help explain the observation of slower recovery of body weight after leptin treatment is terminated. Nevertheless, apoptosis, which has not been well studied in adipocytes, now appears to be an important component in the loss of body fat caused by leptin administration. Better understanding of mechanisms involved in leptin's pro-apoptotic effects on adipose tissue could lead to development of more effective treatments for obesity, and possibly also other disorders of metabolism.

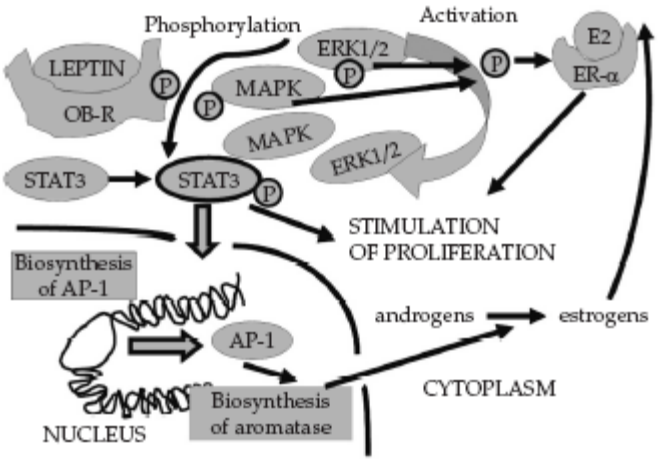

Figure 1. Leptin binds to its receptor OB-R, which becomes phosphorylated by MAPK or ERK. These intracellular kinases probably activate STAT3 by phosphorylation of its tyrosine residue. STAT3 is translocated to the nucleus, where it induces transcription of AP-1 that induces transcription of aromatase. Aromatase changes androgens into estrogens in adipose tissue. Estradiol activates its membrane receptors (ERα) and contributes to proliferation of breast epithelial cells. ERα is sensitized for estrogen stimulation by MAPK and ERK, which can transactivate this receptor independently of estrogens. Apart from induction of aromatase, STAT3 may contribute to cell proliferation by activation of other genes.

OB-R – leptin receptor, P – phosphate residue, AP-1 – transcription activator protein 1, E2 – estradiol, ERα – estrogen receptor α, ERK1/2 – extracellular signal-regulated kinase 1/2, MAPK – mitogen-activated protein kinase

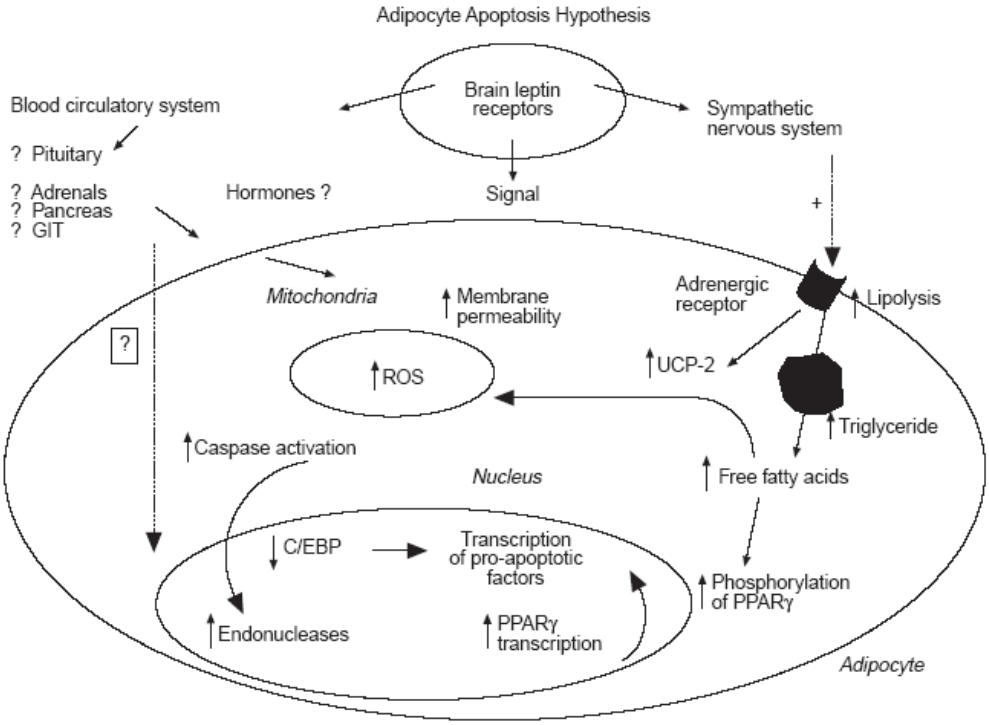

Fig. 4 Adipocyte apoptosis hypothesis. Leptin, acting via brain receptors, causes a hormonal and/or neural signal to be sent to adipose tissue depots. This signal triggers a cascade of events, possibly including increased production and activation of PPARγ, decreased production of C/EBPs, disruption of mitochondrial energy metabolism and increased production of reactive oxygen species, that lead to increased transcription of endonucleases, proteases and phospholipases. These enzymes ultimately result in apoptotic cell death.

Table 1

Anti-apoptotic effects of leptin

Model	Action of leptin	Reference
Hepatocarcinoma	Stimulated cell proliferation; apoptosis was suppressed by down regulating Bax proteins	Chen et al. 2007 (Chen et al., 2007)
Esophageal Adenocarcinoma OE33 cells	Enhanced cell proliferation & inhibition of apoptosis by stimulating EGFR and ERK phosphorylation.	Beales & Ogunwobi, 2007 (Beales and Ogunwobi, 2007)
	Stimulates cell proliferation and inhibits apoptosis via ERK, p38 MAPK, PI-3 kinase/Akt, and JAK-2 dependent activation of COX-2 and PGE2 production.	Ogunwobi et al. 2006 (Ogunwobi et al., 2006)
Cultured Chicken Ovarian cells	Stimulated antiapoptotic proteins Bcl2 and inhibited expression of all markers of cytoplasmic apoptosis (Bax, ASK-1, p53).	Sirotkin & Grossmann, 2007 (Sirotkin and Grossmann, 2007)
Hepatocellular Carcinoma, HepG2 cells	Offers cyto-protection with inhibition of ROS and enhanced activity of Superoxide dismutase (SOD)	Balasubramanian et al. 2007 (Balasubramaniyan et al., 2007)
Rodent Pancreatic Beta Cells, BRIN-BD11 Beta cell-line	Protects cells against apoptosis by upregulating Bcl-2 m RNA expression.	Brown & Dunmore, 2007

	Stimulates STAT3 and STAT5b phosphorylation.	(Brown and Dunmore, 2007) Briscoe et al. 2001 (Briscoe et al., 2001)
Human Colonic Cancer, T(84), HT29/cl.19A, Caco-2 Cell-lines	Enhances proliferation via MAPK and PI3-K pathways. Stimulates cell proliferation and inhibits apoptosis which involves JAK2, PI3 kinase and JNK and activation of STAT3 and AP-1.	Hoda et al. 2007 (Hoda et al., 2007) Ogunwobi & Beales, 2007 (Ogunwobi and Beales, 2007)
Hepatic Stellate Cells (HSCs)	Protects from apoptosis through its interaction with the apoptotic pathway proximal to mitochondrial activation.	Qamar et al. 2006 (Qamar et al., 2006)
Thymus cells	Inhibits apoptosis through a mechanism independent of activation of JAK-2, but depends on the engagement of IRS-1/PI-3 kinase pathway.	Mansour et al. 2006 (Mansour et al., 2006)
Human Eosinophils	Serves as a survival factor for human eosinophils, blocks apoptosis through delayed cleavage of Bax and mitochondrial release of cytochrome c	Conus et al. 2005 (Conus et al., 2005)
Neutrophils	Serves as a survival cytokine for neutrophils by protecting from apoptosis through signaling cascade	

	involving PI3-K and MAPK dependent pathways, delayed cleavage of Bid and Bax proteins.	Bruno et al. 2005 (Bruno et al., 2005)
Neuroblastoma Cells	Inhibits apoptosis via potent down-regulation of caspase-10 and TNF-related apoptosis-inducing ligand.	Russo et al. 2004 (Russo et al., 2004)
Prostate Cancer (DU145, PC3 human prostate cancer cells)	Enhances proliferation and inhibits apoptosis through PI3K and MAPK leptin-receptor-activated pathways.	Somasundar et al. 2004 (Somasundar et al., 2004)

Abrams, J. M., K. White, L. I. Fessler, and H. Steller. 1993. Programmed cell death during drosophila embryogenesis. Development 117: 29-43.

Ahima, R. S., and J. S. Flier. 2000. Leptin. Annu Rev Physiol 62: 413-437.

Almog, B. et al. 2001. Leptin attenuates follicular apoptosis and accelerates the onset of puberty in immature rats. Molecular and Cellular Endocrinology 183: 179-191.

Ambati, S. et al. 2007. Effects of leptin on apoptosis and adipogenesis in 3t3-l1 adipocytes. Biochem Pharmacol 73: 378-384.

Ashkenazi, A., and V. M. Dixit. 1998. Death receptors: Signaling and modulation. Science 281: 1305-1308.

Attoub, S. et al. 2000. Leptin promotes invasiveness of kidney and colonic epithelial cells via phosphoinositide 3-kinase-, rho-, and rac-dependent signaling pathways. Faseb J 14: 2329-2338.

Balasubramaniyan, V., R. Shukla, G. Murugaiyan, R. R. Bhonde, and N. Nalini. 2007. Mouse recombinant leptin protects human hepatoma hepg2 against apoptosis, tnf-alpha response and oxidative stress induced by the hepatotoxin-ethanol. Biochim Biophys Acta 1770: 1136-1144.

Bartness, T. J., G. E. Demas, and C. K. Song. 2002. Seasonal changes in adiposity: The roles of the photoperiod, melatonin and other hormones, and sympathetic nervous system. Exp Biol Med (Maywood) 227: 363-376.

Beales, I. L., and O. O. Ogunwobi. 2007. Leptin synergistically enhances the anti-apoptotic and growth-promoting effects of acid in oe33 oesophageal adenocarcinoma cells in culture. Mol Cell Endocrinol 274: 60-68.

Bhat, G. K. et al. 2006. Influence of a leptin deficiency on testicular morphology, germ cell apoptosis, and expression levels of apoptosis-related genes in the mouse. J Androl 27: 302-310.
Briscoe, C. P., S. Hanif, J. R. Arch, and M. Tadayyon. 2001. Fatty acids inhibit leptin signalling in brin-bd11 insulinoma cells. J Mol Endocrinol 26: 145-154.
Brown, J. E., and S. J. Dunmore. 2007. Leptin decreases apoptosis and alters bcl-2 : Bax ratio in clonal rodent pancreatic beta-cells. Diabetes Metab Res Rev 23: 497-502.
Bruno, A., S. Conus, I. Schmid, and H. U. Simon. 2005. Apoptotic pathways are inhibited by leptin receptor activation in neutrophils. J Immunol 174: 8090-8096.
Caldefie-Chezet, F., A. Poulin, A. Tridon, B. Sion, and M. P. Vasson. 2001. Leptin: A potential regulator of polymorphonuclear neutrophil bactericidal action? J Leukoc Biol 69: 414-418.
Caprio, M. et al. 2003. Ontogenesis of leptin receptor in rat leydig cells. Biol Reprod 68: 1199-1207.
Catalano, S. et al. 2003. Leptin enhances, via ap-1, expression of aromatase in the mcf-7 cell line. Journal of Biological Chemistry 278: 28668-28676.
Catalano, S. et al. 2004. Leptin induces, via erk1/erk2 signal, functional activation of estrogen receptor alpha (era) in mcf-7 cells. J Biol Chem.
Chang, S. et al. 2001. Leptin and prostate cancer. Prostate 46: 62-67.
Chawla, A., and M. A. Lazar. 1994. Peroxisome proliferator and retinoid signaling pathways co-regulate preadipocyte phenotype and survival. Proc Natl Acad Sci U S A 91: 1786-1790.
Chen, C. et al. 2007. Leptin induces proliferation and anti-apoptosis in human hepatocarcinoma cells by up-regulating cyclin d1 and down-regulating bax via a janus kinase 2-linked pathway. Endocr Relat Cancer 14: 513-529.
Chinnaiyan, A. M. et al. 1996. Fadd/mort1 is a common mediator of cd95 (fas/apo-1) and tumor necrosis factor receptor-induced apoptosis. J. Biol. Chem. 271: 4961-4965.
Choi, Y.-H. et al. 2005. Icv leptin effects on spontaneous physical activity and feeding behavior in rats. Endocrinology Annual Meeting.
Conus, S., A. Bruno, and H. U. Simon. 2005. Leptin is an eosinophil survival factor. J Allergy Clin Immunol 116: 1228-1234.
Dagon, Y. et al. 2005. Nutritional status, cognition, and survival: A new role for leptin and amp kinase. J Biol Chem 280: 42142-42148.
Debnath, J., E. H. Baehrecke, and G. Kroemer. 2005. Does autophagy contribute to cell death? Autophagy 1: 66-74.
Della-Fera, M. A., H. Qian, and C. A. Baile. 2001. Adipocyte apoptosis in the regulation of body fat mass by leptin. Diabetes Obes Metab 3: 299-310.
Dieudonne, M. N. et al. 2002. Leptin mediates a proliferative response in human mcf7 breast cancer cells. Biochem Biophys Res Commun 293: 622-628.
Donath, M. Y. et al. 2005. Mechanisms of beta-cell death in type 2 diabetes. Diabetes 54 Suppl 2: S108-113.
Du, C., M. Fang, Y. Li, L. Li, and X. Wang. 2000. Smac, a mitochondrial protein that promotes cytochrome c-dependent caspase activation by eliminating iap inhibition. Cell 102: 33-42.
El-Hefnawy, T., S. Ioffe, and M. Dym. 2000. Expression of the leptin receptor during germ cell development in the mouse testis. Endocrinology 141: 2624-2630.

Elefteriou, F. et al. 2004. Serum leptin level is a regulator of bone mass. Proc Natl Acad Sci U S A 101: 3258-3263.
Elmquist, J. K. 2000. Anatomic basis of leptin action in the hypothalamus. Front Horm Res 26: 21-41.
Elmquist, J. K., C. Bjorbaek, R. S. Ahima, J. S. Flier, and C. B. Saper. 1998. Distributions of leptin receptor mrna isoforms in the rat brain. J Comp Neurol 395: 535-547.
Fleury, C. et al. 1997. Uncoupling protein-2: A novel gene linked to obesity and hyperinsulinemia. Nature Genetics 15: 269-272.
Formigli, L. et al. 2000. Aponecrosis: Morphological and biochemical exploration of a syncretic process of cell death sharing apoptosis and necrosis. J Cell Physiol 182: 41-49.
Frankenberry, K. A., P. Somasundar, D. W. McFadden, and L. C. Vona-Davis. 2004. Leptin induces cell migration and the expression of growth factors in human prostate cancer cells. American Journal of Surgery 188: 560-565.
Frederich, R. C. et al. 1995. Leptin levels reflect body lipid content in mice: Evidence for diet- induced resistance to leptin action. Nat Med 1: 1311-1314.
Friedman, J. M. 2002. The function of leptin in nutrition, weight, and physiology. Nutr Rev 60: S1-14; discussion S68-84, 85-17.
Gainsford, T. et al. 1996. Leptin can induce proliferation, differentiation, and functional activation of hemopoietic cells. Proc Natl Acad Sci U S A 93: 14564-14568.
Garofalo, C., D. Sisci, and E. Surmacz. 2004. Leptin interferes with the effects of the antiestrogen ici 182,780 in mcf-7 breast cancer cells. Clinical Cancer Research 10: 6466-6475.
Garrido, C. et al. 2006. Mechanisms of cytochrome c release from mitochondria. Cell Death Differ 13: 1423-1433.
Gordeladze, J. O., C. A. Drevon, U. Syversen, and J. E. Reseland. 2002. Leptin stimulates human osteoblastic cell proliferation, de novo collagen synthesis, and mineralization: Impact on differentiation markers, apoptosis, and osteoclastic signaling. J Cell Biochem 85: 825-836.
Gregoraszczuk, E. L., and A. Ptak. 2005. In vitro effect of leptin on growth hormone (gh)- and insulin-like growth factor-i (igf-i)-stimulated progesterone secretion and apoptosis in developing and mature corpora lutea of pig ovaries. J Reprod Dev 51: 727-733.
Gullicksen, P. S., M. A. Della-Fera, and C. A. Baile. 2003. Leptin-induced adipose apoptosis: Implications for body weight regulation. Apoptosis 8: 327-335.
Gullicksen, P. S., W. P. Flatt, D. L. Hartzell, and C. A. Baile. 1999. Twenty-one day recovery from leptin administration on energy metabolism in sprague-dawley rats. FASEB Journal 13: A369.
Hacker, G. 2000. The morphology of apoptosis. Cell Tissue Res 301: 5-17.
Halaas, J. L. et al. 1995. Weight-reducing effects of the plasma protein encoded by the obese gene. Science 269: 543-546.
Hardwick, J. C., G. R. Van Den Brink, G. J. Offerhaus, S. J. Van Deventer, and M. P. Peppelenbosch. 2001. Leptin is a growth factor for colonic epithelial cells. Gastroenterology 121: 79-90.
Harvey, J. 2003. Leptin: A multifaceted hormone in the central nervous system. Mol Neurobiol 28: 245-258.

Hoda, M. R. et al. 2007. Leptin acts as a mitogenic and antiapoptotic factor for colonic cancer cells. Br J Surg 94: 346-354.
Hsu, H., J. Xiong, and D. V. Goeddel. 1995. The tnf receptor 1-associated protein tradd signals cell death and nf-kappa b activation. Cell 81: 495-504.
Igney, F. H., and P. H. Krammer. 2002. Death and anti-death: Tumour resistance to apoptosis. Nat Rev Cancer 2: 277-288.
Joza, N. et al. 2001. Essential role of the mitochondrial apoptosis-inducing factor in programmed cell death. Nature 410: 549-554.
Kerr, J. F., A. H. Wyllie, and A. R. Currie. 1972. Apoptosis: A basic biological phenomenon with wide-ranging implications in tissue kinetics. British Journal of Cancer 26: 239-257.
Kumar, S. et al. 2003. Interleukin-1 alpha promotes tumor growth and cachexia in mcf-7 xenograft model of breast cancer. American Journal of Pathology 163: 2531-2541.
Lee, G. H. et al. 1996. Abnormal splicing of the leptin receptor in diabetic mice. Nature 379: 632-635.
Lin, T. et al. 2005. Apoptosis in rat jejunal mucosa is regulated partly through the central nervous system, which controls feeding behavior. J Gastroenterol Hepatol 20: 1285-1291.
Locksley, R. M., N. Killeen, and M. J. Lenardo. 2001. The tnf and tnf receptor superfamilies: Integrating mammalian biology. Cell 104: 487-501.
Maedler, K. et al. 2004. Leptin modulates beta cell expression of il-1 receptor antagonist and release of il-1beta in human islets. Proc Natl Acad Sci U S A 101: 8138-8143.
Majno, G., and I. Joris. 1995. Apoptosis, oncosis, and necrosis. An overview of cell death. Am J Pathol 146: 3-15.
Mansour, E. et al. 2006. Leptin inhibits apoptosis in thymus through a janus kinase-2-independent, insulin receptor substrate-1/phosphatidylinositol-3 kinase-dependent pathway. Endocrinology 147: 5470-5479.
Mantzoros, C. S. et al. 1997. Leptin concentrations in relation to body mass index and the tumor necrosis factor-alpha system in humans. Journal of Clinical Endocrinology and Metabolism 82: 3408-3413.
Mounzih, K., R. Lu, and F. F. Chehab. 1997. Leptin treatment rescues the sterility of genetically obese ob/ob males. Endocrinology 138: 1190-1193.
Naka, T. et al. 1997. Structure and function of a new stat-induced stat inhibitor. Nature 387: 924-929.
Negre-Salvayre, A. et al. 1997. A role for uncoupling protein-2 as a regulator of mitochondrial hydrogen peroxide generation. Faseb J 11: 809-815.
Nicholls, D. G., and R. M. Locke. 1984. Thermogenic mechanisms in brown fat. Physiological Reviews 64: 1-64.
O'Brien S, N., B. H. Welter, and T. M. Price. 1999. Presence of leptin in breast cell lines and breast tumors. Biochem Biophys Res Commun 259: 695-698.
O'Neil, J. S., M. E. Burow, A. E. Green, J. A. McLachlan, and M. C. Henson. 2001. Effects of estrogen on leptin gene promoter activation in mcf-7 breast cancer and jeg-3 choriocarcinoma cells: Selective regulation via estrogen receptors alpha and beta. Mol Cell Endocrinol 176: 67-75.
Ogunwobi, O., G. Mutungi, and I. L. Beales. 2006. Leptin stimulates proliferation and inhibits apoptosis in barrett's esophageal adenocarcinoma cells by cyclooxygenase-2-

dependent, prostaglandin-e2-mediated transactivation of the epidermal growth factor receptor and c-jun nh2-terminal kinase activation. Endocrinology 147: 4505-4516.
Ogunwobi, O. O., and I. L. Beales. 2007. The anti-apoptotic and growth stimulatory actions of leptin in human colon cancer cells involves activation of jnk mitogen activated protein kinase, jak2 and pi3 kinase/akt. Int J Colorectal Dis 22: 401-409.
Okuya, S., K. Tanabe, Y. Tanizawa, and Y. Oka. 2001. Leptin increases the viability of isolated rat pancreatic islets by suppressing apoptosis. Endocrinology 142: 4827-4830.
Paczoska-Eliasiewicz, H. E. et al. 2006. Exogenous leptin advances puberty in domestic hen. Domest Anim Endocrinol 31: 211-226.
Page, K. et al. 2004. Effects of clenbuterol on muscle, adipose tissue and adipocyte apoptosis in myostatin knockout mice. J Anim Sci submitted.
Pelleymounter, M. A. et al. 1995. Effects of the obese gene product on body weight regulation in ob/ob mice. Science 269: 540-543.
Prins, J. B., and S. O'Rahilly. 1997. Regulation of adipose cell number in man. Clin Sci (Lond) 92: 3-11.
Qamar, A. et al. 2006. In vitro and in vivo protection of stellate cells from apoptosis by leptin. Dig Dis Sci 51: 1697-1705.
Qian, H. et al. 1998a. Brain administration of leptin causes deletion of adipocytes by apoptosis. Endocrinology 139: 791-794.
Qian, H. et al. 1998b. Leptin regulation of peroxisome proliferator-activated receptor-gamma, tumor necrosis factor, and uncoupling protein-2 expression in adipose tissues. Biochem Biophys Res Commun 246: 660-667.
Randt, C. T., D. Quartermain, M. Goldstein, and B. Anagnoste. 1971. Norepinephrine biosynthesis inhibition: Effects on memory in mice. Science 172: 498-499.
Russo, V. C., S. Metaxas, K. Kobayashi, M. Harris, and G. A. Werther. 2004. Antiapoptotic effects of leptin in human neuroblastoma cells. Endocrinology 145: 4103-4112.
Satoh, N. et al. 1999. Sympathetic activation of leptin via the ventromedial hypothalamus: Leptin-induced increase in catecholamine secretion. Diabetes 48: 1787-1793.
Schneider, R. et al. 2001. Leptin mediates a proliferative response in human gastric mucosa cells with functional receptor. Horm Metab Res 33: 1-6.
Sierra-Honigmann, M. R. et al. 1998. Biological action of leptin as an angiogenic factor. Science 281: 1683-1686.
Sirotkin, A. V., and R. Grossmann. 2007. Leptin directly controls proliferation, apoptosis and secretory activity of cultured chicken ovarian cells. Comp Biochem Physiol A Mol Integr Physiol 148: 422-429.
Slee, E. A., C. Adrain, and S. J. Martin. 2001. Executioner caspase-3, -6, and -7 perform distinct, non-redundant roles during the demolition phase of apoptosis. J Biol Chem 276: 7320-7326.
Somasundar, P. et al. 2004. Prostate cancer cell proliferation is influenced by leptin. J Surg Res 118: 71-82.
Somasundar, P., A. K. Yu, L. Vona-Davis, and D. W. McFadden. 2003. Differential effects of leptin on cancer in vitro. J Surg Res 113: 50-55.
Sperandio, S. et al. 2004. Paraptosis: Mediation by map kinases and inhibition by aip-1/alix. Cell Death Differ 11: 1066-1075.
Suzuki, Y. et al. 2001. A serine protease, htra2, is released from the mitochondria and interacts with xiap, inducing cell death. Mol Cell 8: 613-621.

Tartaglia, L. A. 1997. The leptin receptor. J Biol Chem 272: 6093-6096.
Tartaglia, L. A. et al. 1995. Identification and expression cloning of a leptin receptor, ob-r. Cell 83: 1263-1271.
Tessitore, L. et al. 2004. Adipocyte expression and circulating levels of leptin increase in both gynaecological and breast cancer patients. Int J Oncol 24: 1529-1535.
Trayhurn, P., and J. H. Beattie. 2001. Physiological role of adipose tissue: White adipose tissue as an endocrine and secretory organ. Proc Nutr Soc 60: 329-339.
Wajant, H. 2002. The fas signaling pathway: More than a paradigm. Science 296: 1635-1636.
Wang, J., and I. L. Campbell. 2002. Cytokine signaling in the brain: Putting a socs in it? J Neurosci Res 67: 423-427.
White, D. W. et al. 2000. Identification of leptin-induced transcripts in the mouse hypothalamus. Diabetes 49: 1443-1450.
Yamagishi, S. I. et al. 2001. Leptin induces mitochondrial superoxide production and monocyte chemoattractant protein-1 expression in aortic endothelial cells by increasing fatty acid oxidation via protein kinase a. J Biol Chem 276: 25096-25100.
Yoshitomi, H., K. Yamazaki, S. Abe, and I. Tanaka. 1998. Differential regulation of mouse uncoupling proteins among brown adipose tissue, white adipose tissue, and skeletal muscle in chronic á3-adrenergic receptor agonist treatment. Biochemical and Biophysical Research Communications 253: 85-91.
Zhang, H. H., S. Kumar, A. H. Barnett, and M. C. Eggo. 1999. Intrinsic site-specific differences in the expression of leptin in human adipocytes and its autocrine effects on glucose uptake. J Clin Endocrinol Metab 84: 2550-2556.