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INSIDE

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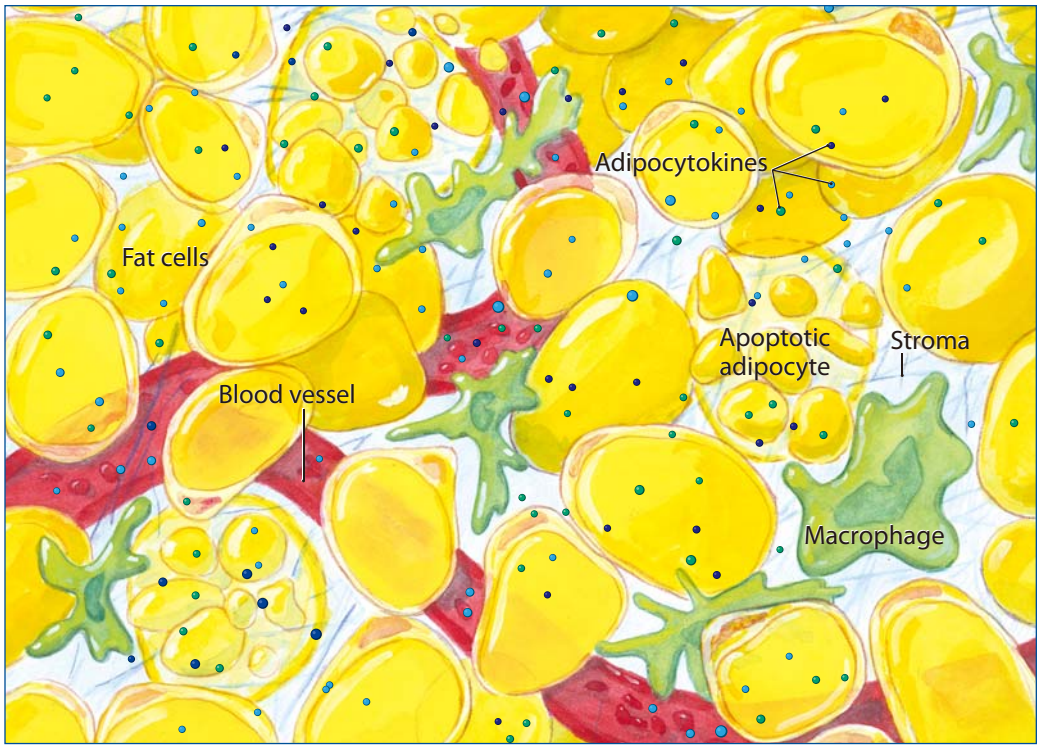


Obesity is characterized by an increase in adipocyte size and number, changes in the levels of adipocytokine secretion, and recruitment of macrophages that release pro-inflammatory cytokines. Altered circulating levels of adipocytokines, released by adipocytes themselves, associated macrophages, or stromal cells can have deleterious effects on cellular proliferation, apoptosis, invasive growth, or angiogenesis. This suggests that adipocytokines may be involved in the growth and/or metastasis of obesity-related cancers. [Figure adapted from Tilg, H. *et al.* (2006) Nat. Rev. Immunol. **6**:772]

Obesity, Adipocytokines, & Breast Cancer

Adipose tissue is an active endocrine organ, secreting fatty acids, and peptide hormones or cytokines, collectively called adipocytokines. These biologically active factors act locally or peripherally to influence multiple processes, including glucose and fatty acid metabolism, insulin sensitivity, adipocyte differentiation, inflammation, and the immune response. Accumulating evidence suggests that obesity, a condition characterized by an increase in adipocyte size and number, altered adipocytokine secretion, and increased angiogenesis, is one of the risk factors for certain types of cancer, including postmenopausal breast cancer, and endometrial cancer.[1] While the cause of these obesity-related cancers has been primarily ascribed to excess estrogen production by adipose tissue, they have also been speculated to be due, in part, to changes in the levels of adipocytokines secreted by adipocytes, infiltrating macrophages, or associated stromal cells.[2] Changes in adipocytokine levels can affect cell proliferation, apoptosis, invasive growth, and angiogenesis. Although numerous adipocytokines have been identified, the effects of only a few in promoting or inhibiting mammary tumor growth have been extensively studied to date. These include leptin, hepatocyte growth factor (HGF), and adiponectin.

Obese individuals typically have elevated levels of circulating leptin, a hormone associated with appetite suppression and energy expenditure. Identifying a causal link between elevated leptin levels and obesity-related postmenopausal breast cancers has been complicated by conflicting results, but multiple studies now suggest that leptin has proliferative and pro-angiogenic effects that may promote mammary tumorigenesis. Leptin has been shown to increase the proliferation of human breast cancer cell lines expressing the leptin receptor (Ob-R), including MCF-7, ZR75-1, and the estrogen-receptor negative, HTB-26 cells.[3,4] Cleary *et al.* took these observations one step further by establishing a direct link between leptin signaling and breast cancer development *in vivo*. Their results demonstrated that obese mice deficient in either leptin or the leptin receptor, and overexpressing TGF-α, have a reduced occurrence of oncogene-induced mammary tumors, compared to lean control mice.[5,6] Significantly, it was found that

Continued on page 5

Osteopontin and Syndecan-4 in Liver Inflammation

Osteopontin (OPN) is a secreted molecule in the SIBLING family of non-collagenous matricellular proteins. It is a 45 - 75 kDa acidic protein that is variably modified in a tissue specific manner by glycosylation, sulfation, phosphorylation, and transglutamination. OPN plays a major role in bone mineralization, kidney stone formation, cell adhesion and migration, and inflammation. It is upregulated in response to inflammation, cancer, and tissue damage. The central region of OPN contains RGD and non-RGD binding sites for multiple integrins. Adjacent to the RGD motif is the sequence SVVYGLR (SLAYGLR in mouse) which serves as a cryptic binding site for additional integrins: it is masked in full length OPN but is exposed following OPN cleavage by thrombin.[1-5] The N-terminal fragment of OPN ending in the SVVYGLR motif is more avid than full length OPN at mediating cell adhesion and migration.[1-3]

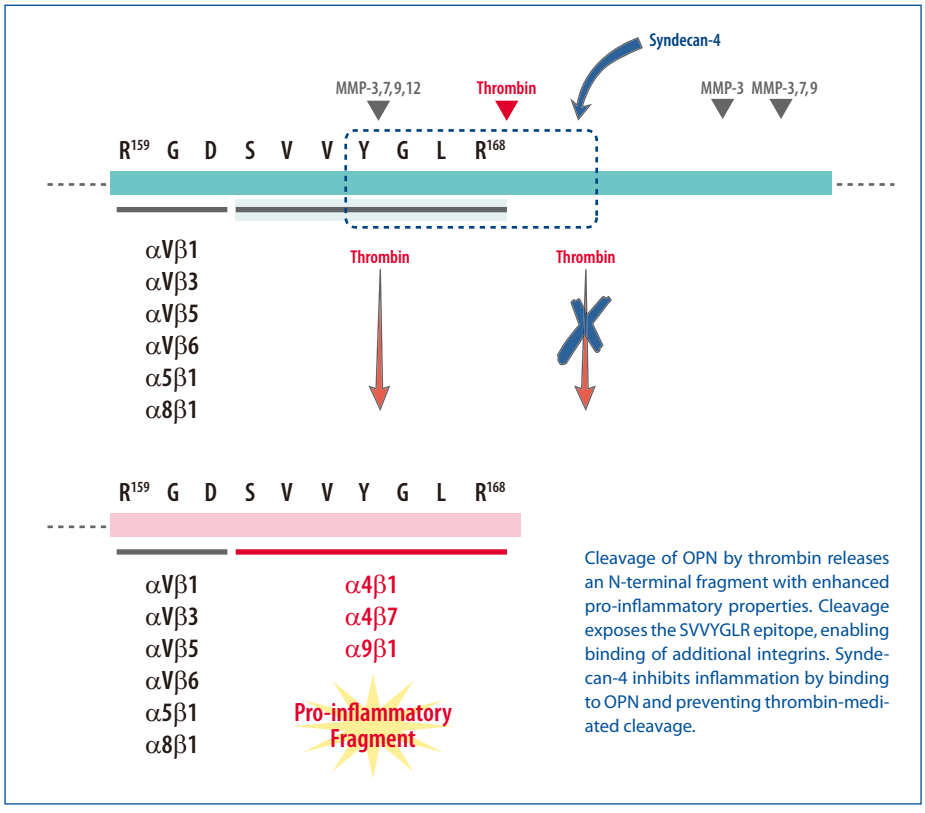
OPN can also be cleaved by MMP-3, -7, -9, and -12 within the SVVYGLR motif and at sites closer to the C-terminus.[6,7] MMP-mediated cleavage of OPN at the first site has differential effects on binding to particular integrins.[3,6,8] Cleavage at the more C-terminal MMP sites has not been shown to affect integrin binding, although complete digestion of OPN by MMP-9 releases a 5 kDa fragment which promotes CD44-dependent metastasis in hepatocellular carcinoma.[7] It has not been determined if cleavage by thrombin or MMPs can be sequential or if digestion by one enzyme prevents further digestion by a second.

Recently, Kon *et al.* identified the proteoglycan Syndecan-4 as a novel OPN binding partner and regulator. These authors demonstrated that the heparan sulfate moiety of Syndecan-4 interacts with the heparin-binding domain (HBD) of OPN at the thrombin cleavage site, but not at a second HBD near the C-terminus.[9] Formation of the OPN/Syndecan-4 complex likely represses OPN proteolysis by obstructing the thrombin cleavage site.[9] Syndecan-4 binding to OPN blocks the binding of integrin α4β1, which recognizes the cryptic SVVYGLR epitope and integrin αVβ3, which recognizes the RGD motif.[9]

In a mouse model of hepatitis, concanavalin A-induced granuloma formation and hepatocyte cell death requires the secretion of OPN by resident CD1d-restricted natural killer T (NKT) cells.[10,11] These cells are responsible for the bulk of the OPN elevation seen in the liver and serum. The fraction of OPN that is cleaved by thrombin is critical for neutrophil migration and activation, and for further stimulation of OPN release by NKT cells.[11] Both NKT cells and neutrophils express integrin α4β1, which binds the cryptic epitope. Blocking antibodies to the SLAYGLR motif prevent OPN-induced hepatocyte necrosis and elevation of serum alanine aminotransferase, a physiological indicator of liver damage.[11]

Using the Concanavalin A-induced hepatitis model, Kon *et al.* showed that Syndecan-4 provides protection against OPN mediated toxicity. Compared to wild type mice, Syndecan-4 knockout mice sustained more extensive liver damage in terms of histology and serum liver enzyme levels.[9] Circulating thrombin-cleaved OPN was elevated as previously described by Diao *et al.*, and antibody neutralization of the N-terminal fragment improved the tissue and serological indicators of liver damage.[9] The increased shedding of Syndecan-4 in this model may constitute a protective response, consistent with the observation that exogenous soluble Syndecan-4 blocked inflammatory cell infiltration into the liver and hepatocyte necrosis.[9]

The functions of OPN may be determined by the relative abundance of different forms and modifications. The ability of Syndecan-4 to block the generation of pro-inflammatory OPN fragments represents one of potentially many mechanisms to control the prevalence of these forms.



Cleavage of OPN by thrombin releases an N-terminal fragment with enhanced pro-inflammatory properties. Cleavage exposes the SVVYGLR epitope, enabling binding of additional integrins. Syndecan-4 inhibits inflammation by binding to OPN and preventing thrombin-mediated cleavage.

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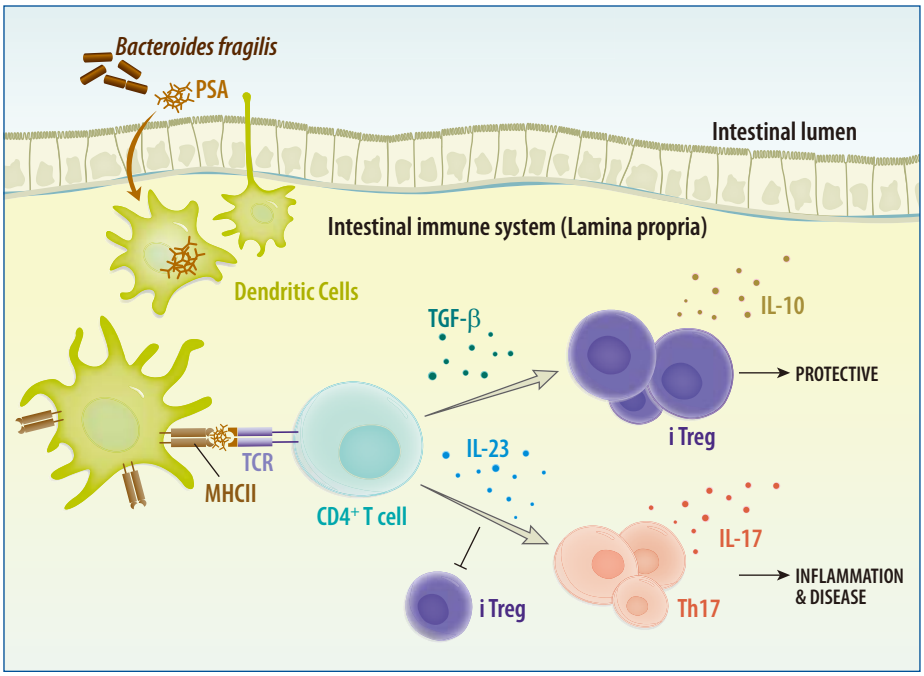
An Active Role for Microorganisms in Controlling Intestinal Inflammation

The innate and adaptive immune systems in the intestines are uniquely adapted to a milieu that occurs nowhere else in the body. Microorganisms within the intestines are said to outnumber cells in the body by 10-100 fold.[1] These organisms present the problem of how to discriminate between commensals (to which there should be no immune response) and pathogens (to which there should be an immediate immune response).[1] When discrimination between these microbes breaks down, inflammatory bowel diseases (IBD) such as Crohn's disease and ulcerative colitis occur. The incidence of IBD has increased over the past 30 years, especially in developed countries. One attractive theory to explain the increase is the "hygiene hypothesis", which postulates that increased antibiotic use and cleaner home environments may promote over-reaction to certain commensals, sometimes called pathobionts, and result in inflammatory diseases such as IBD.[2] It is said that immune responses in the normal intestine are in a constant state of controlled inflammation. New evidence suggests that, while pathobionts may overwhelm control mechanisms, other commensals, called symbionts, may be active partners in establishing and maintaining this control.[2,3]

Normal mice maintained from birth in a germ-free environment are systemically deficient in $CD4^+CD45^{low}$ T lymphocytes, which are known to include regulatory T cells critical in controlling intestinal inflammation.[2,3] Colonization with *Bacteroides fragilis*, a prominent component of mammalian intestinal flora, is by itself able to correct this deficiency as well as promote balance between Th1 and Th2 populations.[2,4] Surprisingly, the benefit of *B. fragilis* relies on, and can be substituted by, the zwitterionic capsular polysaccharide PSA.[2,4] Previous data has shown that dendritic cells (DC), which can extend processes from the lamina propria to the intestinal lumen,[1] can take up PSA by a TLR2-dependent mechanism, then process and present it on MHC Class II molecules.[5] Its presentation promotes T cell production of IL-10, a key anti-inflammatory molecule in the intestinal immune system that can be produced by regulatory T cells.[2] PSA promotes control over production of critical cytokines in the pathogenesis of human IBD, including TNF-α and IL-23, as well as amelioration of IBD symptoms such as colonic epithelial hyperplasia, leukocyte infiltration, inflammation, and wasting. Either of two commonly used mouse models supports these findings. In the first model, the chemical TNBS was used to induce a Th17 cell-mediated inflammatory disease. IL-17 and TNF-α were elevated in these diseased animals, but not in PSA-protected animals, which also showed less intestinal pathology and inflammation.[2] In the second model, inflammation was promoted by a pathobiont (*Helicobacter hepaticus*) in a host predisposed to colitis. PSA provided protection from inflammatory reactions and a decrease in TNF-α.[2] The ability of T cells to produce IL-10 was critical for the reduced inflammatory effect of PSA.[2]



Polysaccharide A (PSA) is taken up by lamina propria dendritic cells, processed, and presented to naïve $CD4^+$ T cells. In the presence of activated TGF-β, these cells can become induced regulatory T cells (iTreg). Production of IL-10 by these and other T-lineage cells promotes control of immune activation. IL-23 inhibits control by Treg, and promotes expansion of inflammatory Th17 cells. For simplicity, many other pro- and anti-inflammatory mechanisms present in the intestines are not shown. (Figure adapted from reference #10)

Other intestinal microorganisms also appear to have a positive effect on the intestinal immune system, and have been proposed as probiotic treatments for human IBD.[6] *In vitro* studies indicate that their modes of action might be similar to *B. fragilis*. Cells from human mesenteric lymph nodes that drain inflamed intestines secrete more anti-inflammatory cytokines (IL-10, TGF-β) when stimulated with probiotic variants of *Bifidobacterium* or *Lactobacillus*, but more pro-inflammatory cytokines (TNF-α, IL-12) when stimulated with pathogenic *Salmonella*.[7] Specific IL-10 secretion is also seen upon stimulation of peripheral blood mononuclear cells from ulcerative colitis patients with heat-killed variants of *Bifidobacterium sp*.[8] Early clinical trials of these and other potential probiotics have been encouraging.[6] Since *Bacteroides* and *Lactobacillus* are genera that show decreased representation in the intestines of many IBD patients,[9] it is intriguing to speculate that symbiont colonization may be deficient in these patients. It will also be interesting to see whether, like PSA, polysaccharides expressed by other probiotics play an active role in controlling intestinal immune responses.

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α-Synuclein-based Model for Studying Parkinson's Disease Pathology

Parkinson's disease (PD) is a multicentric, progressive neurodegenerative disorder that strikes approximately 1% of people aged 65 and older.[1-2] Clinically, it is characterized by severe motor symptoms including muscular rigidity, uncontrollable resting tremor, and bradykinesia, along with secondary symptoms such as postural instability, cognitive dysfunction (dementia, psychosis), sleep abnormalities, and mood disorders (depression, anxiety).[1-2] The pathophysiology of PD results from the progressive and selective loss of dopaminergic neurons in the substantia nigra pars compacta,[3] with the consequent depletion of dopamine in its striatal projections, and other brainstem regions. This leads to disruption of the cerebral neuronal systems responsible for motor function.[1,4] In addition, the PD brain is characterized by the presence of cytoplasmic and neuritic fibrillar α-synuclein inclusions (known as Lewy bodies and Lewy neurites, respectively) in the surviving dopaminergic neurons and other affected areas of the CNS.[1]

The precise pathogenesis of the majority of PD cases is unclear, but genetic mutations in the gene for α-synuclein (A53T, A30P, and E46K)[5-6] and overexpression of α-synuclein gene have been associated with familial forms of PD.[2] PD characterized by α-synuclein containing Lewy bodies accounts for >90% of sporadic Parkinsonian cases.[2] The mechanism of α-synuclein-induced toxicity is still being investigated, but may be related to the propensity of normal α-synuclein and mutated forms to self-aggregate at higher concentrations, producing fibrils with amyloid-like cross-beta conformation.[1] Zhang *et al.* propose that nigral neuronal damage may release aggregated α-synuclein into the substantia nigra, activating microglia and the subsequent production of pro-inflammatory mediators and ROS (reactive oxygen species). These factors contribute to persistent and progressive nigral degeneration in PD.[7]

Animal models of PD are essential tools for identifying novel therapeutic targets and testing potential therapies. Until recently, the field has been dominated by toxin-based models.[8-9] These models are valuable for understanding what happens when nigrostriatal dopaminergic cells are lost, for testing dopamine replacement therapies, and for identifying interventions to reduce lesion size.[9] Nevertheless, these approaches are valuable only when it is assumed that the mechanism of action of these toxins is germane to the pathophysiological process occurring in PD.[9] But this is not necessarily true. Evidence exists that some of these toxins cause dopaminergic cell loss in humans (e.g. the mitochondrial toxin, MPTP) or increase the risk of PD after long-term exposure, but there is no clear evidence that the mechanisms are the same.[9] As a result, these models do not display the full pathology and progression seen in PD.[1] Schneider *et al.* have developed an *in vitro* model based on human progenitor cells.[10] Their model convincingly shows the cytotoxicity of α-synuclein, although it too has limitations. Ethical concerns and technical hurdles still limit the derivation of these cells, and like the toxin-based models, they do not show the time-dependent pathogenesis characterized by PD.[10]

Recchia *et al.* now describe and characterize an animal model that more faithfully reproduces α-synuclein-induced neurotoxicity and human PD pathology.[1] Their model is based on the sterotaxic injection of the A30P mutated form of α-synuclein fused to a protein transduction domain (TAT) into the right substantia nigra pars compacta of rat.[1] In their study, the infusion of TAT-α-synuclein A30P induced a significant loss in dopaminergic neurons (26%), which was followed by a time-dependent impairment of motor function.[1] Compared to chemical neurotoxin-based animal models of PD, the α-synuclein-based PD animal model more clearly mimics the early stages and slow development of the human disease. Therefore, this model should prove valuable in evaluating specific aspects of PD pathogenesis *in vivo* and in developing new therapeutic strategies.[1]

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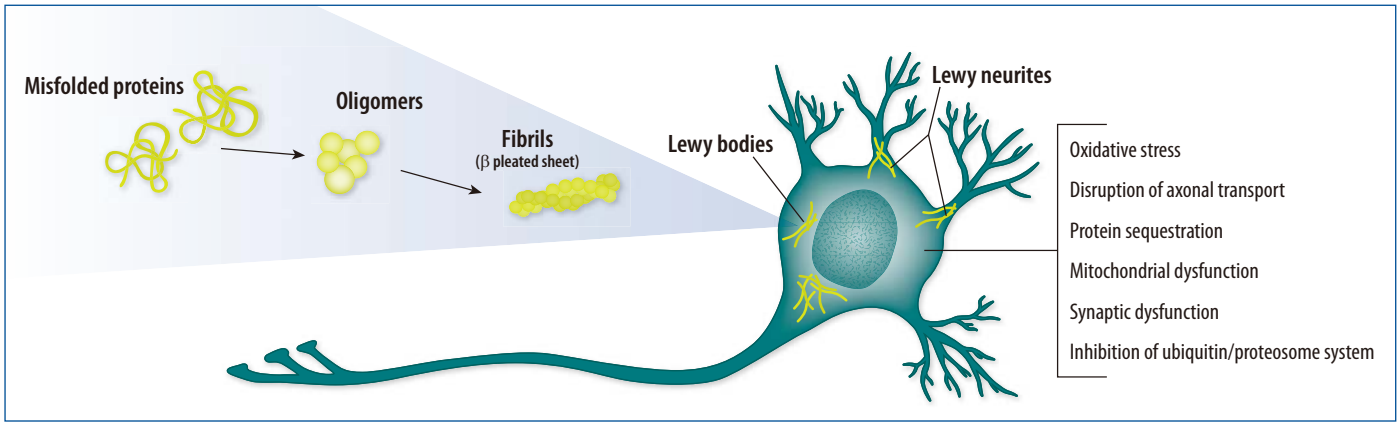

Misfolded α-synuclein proteins are converted into pathological oligomers and higher order aggregates that form fibrils and deposit into Lewy bodies and Lewy neurites in affected neurons of the PD brain. Several consequences of these fibrillar deposits of α-synuclein have been proposed and are indicated. (Figure adapted from reference #2).

obese mice lacking leptin, or the leptin receptor also display defects in mammary gland morphogenesis, implicating leptin in normal mammary tissue development.[7] A subsequent study demonstrated that increasing concentrations of a leptin receptor antagonist could also slow tumor development in mice.[8] This study showed that leptin signaling promotes the expression of vascular endothelial growth factor (VEGF) and VEGF R2, indicating that leptin may stimulate tumor-related angiogenesis.[8] In addition to acting through its own receptor, leptin has been shown to induce ligand-independent activation of estrogen receptor α in MCF-7 breast cancer cells.[9] It also promotes aromatase activity in these cells which is a key enzyme required for estrogen biosynthesis.[10] Taken together, these studies suggest that leptin signaling through its own receptor, or through activation of estrogen receptor signaling pathways, promotes cell proliferation and angiogenesis. Obesity-related leptin hypersecretion may contribute to the excess estrogen production, unregulated cell growth, and increased angiogenesis associated with mammary tumorigenesis.

Adipose tissue is a major source of HGF, another adipocytokine that like leptin, is elevated in obesity and promotes both cell proliferation and angiogenesis. Expression of a constitutively active form of the HGF receptor, also known as c-Met, in transgenic mice induces mammary tumor formation, indicating that loss of HGF:c-Met regulation may be involved in breast cancer pathogenesis.[11] In addition, HGF signaling was demonstrated to regulate the loss of tumor mass adhesion, and breakdown of the extracellular matrix, suggesting that HGF plays a central role in tumor cell metastasis.[12] Significantly, HGF has been shown to increase the migration and invasiveness of MDA-MB-231 human breast cancer cells *in vitro*.[13] HGF also stimulates VEGF-dependent or independent tumor angiogenesis,[14] indicating that it may help sustain tumor growth through its ability to induce the formation of new blood vessels. Several other adipocytokines promote angiogenesis as well, including heparin-binding epidermal growth factor-like growth factor (HB-EGF), tumor necrosis factor α (TNF-α), and IL-6 (see Table), implying that increases in the levels of theseadipocytokines may also be associated with obesity-related cancers.

Molecules Secreted by Adipose Tissue	Role
Acylation-stimulating protein	Metabolic regulator
Adiponectin/Acrp30	Metabolic regulator; Inhibits angiogenesis; Anti-inflammatory
Agouti protein	Adipocyte lipid metabolism and differentiation
Apelin	Regulates cardiovascular functions; Up-regulated in obesity
Chemerin/Tig-2	Chemoattractant protein; Adipocyte differentiation; Metabolic regulator
Complement Factor D/Adipsin	Serine protease; Immune response
FABP4/AFABP	Fatty acid binding protein; Lipid transport
HB-EGF	Negatively regulates adipogenesis; Promotes angiogenesis
HGF	Mitogenic and angiogenic growth factor
IGF-I R	Receptor tyrosine kinase; Pre-adipocyte differentiation
IL-6	Inflammatory cytokine; Promotes angiogenesis; Regulates insulin sensitivity
IL-8/CXCL8	Pro-inflammatory chemokine
Intelectin-1/Omentin	Regulates insulin sensitivity
Leptin	Metabolic regulator; Promotes proliferation and angiogenesis
Lipocalin 2/NGAL	Antagonist of inflammatory adipocytokine secretion
MIF	Inflammatory cytokine
NGF	Neurotrophic growth factor; Inflammatory response
PBEF-1/Visfatin	Insulin mimicking and pro-inflammatory effects; Up-regulated in obesity
PPARγ/NRIC3	Nuclear regulator of metabolism
Pref-1/DLK-1/FA1	Preadipocyte membrane protein; Inhibits adipogenesis
Retinol-binding Protein 4/RBP4	Regulates insulin sensitivity
Resistin/ADSF	Metabolic regulator; Pro-inflammatory
Serpin A8/Angiotensinogen	Serine protease inhibitor; Promotes adipose tissue growth
Serpin A12/Vaspin	Serine protease inhibitor; Regulates insulin sensitivity
Serpin E1/PAI-1	Serine protease inhibitor; Extracellular matrix remodeling
Serum amyloid A1/SAA1	Apolipoprotein; Low-grade inflammation
TGF-β	Inhibitor of adipocyte differentiation
TNF-α/TNFSF1A	Inflammatory cytokine; Regulates leptin; Promotes angiogenesis; Regulates insulin sensitivity
TNF RI/TNFRSF1A	Cytokine receptor; Pro-inflammatory
VEGF	Angiogenic growth factor

This list represents some of the physiologically active molecules produced by the adipose tissue according to what has been reported in the scientific literature and is not intended to be comprehensive or definitive.

Adiponectin levels, unlike leptin and HGF levels, are reduced in obesity and in breast cancer patients. In fact, the concentration of circulating adiponectin is inversely associated with the risk for developing postmenopausal breast cancer.[15] Recent studies have demonstrated that adiponectin inhibits the growth of breast cancer cell lines (MDA-MB-231 and MCF-7 cells) expressing the two adiponectin receptors, AdipoR1 and AdipoR2.[16,17] Brakenhielm, *et al.* have also shown that adiponectin acts as a negative regulator of angiogenesis *in vivo* by inducing endothelial cell apoptosis.[18] Significantly, the same study demonstrated that adiponectin could directly inhibit tumor growth and reduce neovascularization *in vivo*.[18] Therefore, the reduction in the levels of adiponectin associated with obesity may promote cancer growth by way of a decrease in anti-angiogenic and anti-proliferative activities.

Characteristics of obesity, such as changes in adipocyte size and number and the recruitment of pro-inflammatory mediators lead to changes in adipocytokine secretion which can increase the risk of developing certain forms of cancer. Since these physiologically active molecules seem to play important roles in cell proliferation and angiogenesis, determining the mechanisms by which adipocytokines act locally and peripherally is critical to understanding how they may be involved in promoting or inhibiting tumor growth and metastasis.

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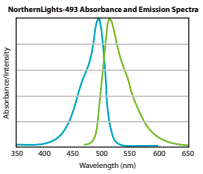
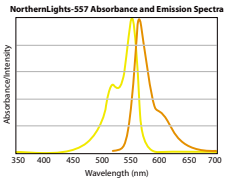
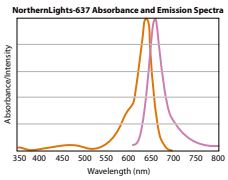
TECHNICAL NOTE:
Using NorthernLights™ Fluorescent Secondary Antibodies for Neuronal Tracing

Immunofluorescence histochemistry is a powerful tool frequently used in neuroscience for qualitative and quantitative morphological and anatomical studies. Because samples are subjected to prolonged examination under conventional fluorescence microscopes and multiple laser-scanning confocal microscopes, it is critical for fluorescent tags to have a low susceptibility to photobleaching. Conjugates comprised of bright fluorescent tags and highly specific secondary antibodies that don't cross-react with irrelevant cell and tissue targets are optimal. Our line of NorthernLights™ fluorescent secondary reagents include anti-mouse, anti-goat, anti-rabbit, anti-sheep, and anti-rat affinity purified antibodies, and streptavidin conjugates. These fluorochromes are bright, resistant to photobleaching, and stable in tissue clearing agents, including alcohol and xylenes. Spectral characteristics of NorthernLights conjugates are comparable to other common fluorochromes allowing use with both conventional fluorescence and laser confocal microscopes (Table 1).

High fluorescence output and resistance to photobleaching make NorthernLights conjugates the reagents of choice for the detection of low abundant cell and tissue antigens. NorthernLights streptavidin conjugates can also be used for the neuronal tracing of biocytin-filled neurons. Labeled neurons in tissue slices can be viewed with exceptional resolution that details fine morphological structures in axons, dendrites, and dendritic spines. This allows for accurate 3D neuronal reconstruction and image analysis (Figure 1).

Table 1

NorthernLights™ Secondary Reagents	Cat. #	Abs/Em Maxima	Laser (Ex)	Fluorochromes with Comparable Spectra (Abs/Em)
NorthernLights-493 anti-Sheep IgG	NL012	493/514 	Argon (488)	FITC (492/520) Cy™5 (489/506) Alexa Fluor® 488 (494-519)
NorthernLights-493 anti-Rabbit IgG	NL006			
NorthernLights-493 anti-Mouse IgG	NL009			
NorthernLights-493 anti-Goat IgG	NL003			
NorthernLights-493 anti-Rat IgG	NL015			
NorthernLights-493 Streptavidin conjugate	NL997			
NorthernLights-493 anti-Chicken IgY	NL018			
NorthernLights-557 anti-Sheep IgG	NL010	557/574 	Krypton (568) HeNe (543)	Phycoerythrin (565/575) Rhodamine™ Red X (570/590) Cy™3 (548/562)
NorthernLights-557 anti-Rabbit IgG	NL004			
NorthernLights-557 anti-Mouse IgG	NL007			
NorthernLights-557 anti-Goat IgG	NL001			
NorthernLights-557 anti-Rat IgG	NL013			
NorthernLights-557 Streptavidin conjugate	NL999			
NorthernLights-557 anti-Chicken IgY	NL016			
NorthernLights-637 anti-Sheep IgG	NL011	637/658 	HeNe (633)	Allophycocyanin (645-660) Alexa Fluor® 647 (650-668) Cy™5 (650/670)
NorthernLights-637 anti-Rabbit IgG	NL005			
NorthernLights-637 anti-Mouse IgG	NL008			
NorthernLights-637 anti-Goat IgG	NL002			
NorthernLights-637 anti-Rat IgG	NL014			
NorthernLights-637 Streptavidin conjugate	NL998			
NorthernLights-637 anti-Chicken IgY	NL017			

Neuronal Tracing

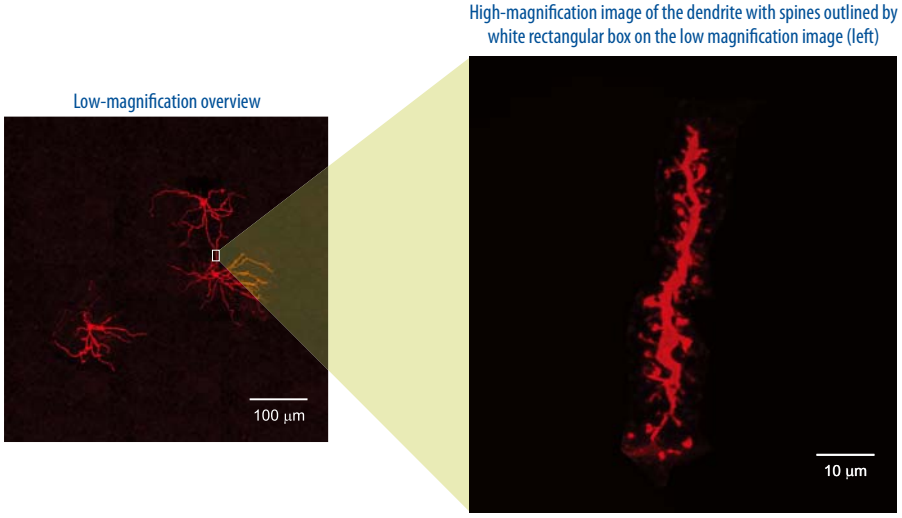


Figure 1. Neuronal tracing of medium spiny neurons in mouse nucleus accumbens using NorthernLights-557 Streptavidin conjugate. Low-magnification image (left) represents a Z-stack projection of 82 images collected in 1 micron steps. High-magnification image of the dendrite with spines (right) represents a Z-stack projection of 48 images collected in 0.1 micron steps.

Procedure for the neuronal tracing technique:

1. 240 micron thick sagittal sections through the nucleus accumbens from C57/Bl6 mice were obtained.
2. Medium spiny neurons (MSNs) were injected with biocytin in cesium gluconate internal pipette solution.
3. Slices were fixed, washed, and incubated with 1 µg/mL of R&D Systems NL-557 Streptavidin (Catalog # NL999) to visualize biocytin-injected MSNs.

Acknowledgement : We express our thanks to Dr. Mark J. Thomas and researchers Said Kourrich and Patrick E. Rothwell (Department of Neuroscience, University of Minnesota, Minneapolis, MN 55455, USA) for providing brain slices with biocytin-filled neurons.

For more information visit our website at **www.RnDSystems.com/go/NorthernLights**

RECENT CITATIONS: **Neurodegenerative Disorders**

1. Rentzos, M. *et al.* (2007) Circulating Interleukin-15 and RANTES chemokine in Parkinson's Disease. Acta. Neurol. Scand. **116**:374.

Human IL-15 Quantikine® ELISA Kit
(Catalog # D1500)
Sample: Human serum
Application: ELISA

Human CCL5/RANTES Quantikine ELISA Kit
(Catalog # DRN00B)
Sample: Human serum
Application: ELISA

2. Gasmi, M. *et al.* (2007) AAV2-mediated delivery of human neurturin to the rat nigrostriatal system: long-term efficacy and tolerability of CERE-120 for Parkinson's Disease. Neurobiol. Dis. **27**:67.

Human Neuritin Polyclonal Antibody
(Catalog # AF283)
Sample: Rat brain
Application: Immunohistochemistry

Human GDNF Polyclonal Antibody
(Catalog # AF-212-NA)
Sample: Rat brain
Application: Immunohistochemistry

3. Parish, C.L. *et al.* (2008) Wnt5a-treated midbrain neural stem cells improve dopamine cell replacement therapy in Parkinsonian mice. J. Clin. Invest. **118**:149.

Recombinant Human FGF basic
(Catalog # 233-FB)
Sample: Mouse ventral midbrain neural stem cells
Application: Neurosphere development

Recombinant Mouse FGF-8b
(Catalog # 423-F8)
Sample: Mouse ventral midbrain neural stem cells
Application: Neurosphere development

Recombinant Mouse Sonic Hedgehog
(Catalog # 461-SH)
Sample: Mouse ventral midbrain neural stem cells
Application: Neurosphere development

Recombinant Human BDNF
(Catalog # 248-BD)
Sample: Mouse ventral midbrain neural stem cells
Application: Neurosphere development

Recombinant Mouse Wnt-5a
(Catalog # 645-WN)
Sample: Mouse ventral midbrain neural stem cell-derived neurospheres
Application: Dopaminergic neuron differentiation

Recombinant Mouse Dkk-1
(Catalog # 1765-DK)
Sample: Mouse ventral midbrain neural stem cell-derived neurospheres
Application: Blocking Wnt-5a induced dopaminergic neuron differentiation

Mouse Wnt-5a Polyclonal Antibody
(Catalog # AF645)
Sample: Mouse ventral midbrain neural stem cell-derived neurospheres
Applications: Neutralization and Western blot

4. Marchionini, DM. *et al.* (2007) Role of heparin binding growth factors in nigrostriatal dopamine system development and Parkinson's disease. Brain Res. **1147**:77.

Human Midkine Polyclonal Antibody
(Catalog # AF-258-PB)
Sample: Rat brain homogenate
Application: Western blot

Human Pleiotrophin (PTN) Polyclonal Antibody
(Catalog # AF-252-PB)
Sample: Rat brain homogenate
Application: Western blot

5. Kalkonde, Y.V. *et al.* (2007) Chemokines in the MPTP model of Parkinson's disease: absence of CCL2 and its receptor CCR2 does not protect against striatal neurodegeneration. Brain Res. **1128**:1.

Mouse CCL2/JE/MCP-1 Polyclonal Antibody
(Catalog # AF-479-NA)
Sample: Mouse brain
Application: Immunohistochemistry

Mouse CCL3/MIP-1α Polyclonal Antibody
(Catalog # AF-450-NA)
Sample: Mouse brain
Application: Immunohistochemistry

6. de Jong, E.K. *et al.* (2008) Expression of CXCL4 in microglia *in vitro* and *in vivo* and its possible signaling through CXCR3. J. Neurochem. **105**:1726.

Mouse CXCL4/PF4 DuoSet® ELISA Development System
(Catalog # DY595)
Sample: Mouse primary microglial supernates
Application: ELISA

Recombinant Mouse CXCL4/PF4
(Catalog # 595-P4)
Sample: Mouse primary microglial cells
Application: Bioassay- chemotaxis

Mouse CXCR3 Phycoerythrin-labeled Monoclonal Antibody
(Catalog # FAB1685P)
Sample: Mouse primary microglial cells
Application: Flow cytometry

7. Qin, W. *et al.* (2008) Down-regulation of insulin-degrading enzyme by presenilin 1 V97L mutant potentially underlies increased levels of amyloid beta 42. Eur. J. Neurosci. **27**:2425.

Human Insulysin/IDE Polyclonal Antibody
(Catalog # AF2496)
Sample: Human SH-SY5Y neuroblastoma cell line transfected with P1 V97L lysates
Application: Western blot

8. Basso, A.S. *et al.* (2008) Reversal of axonal loss and disability in a mouse model of progressive multiple sclerosis. J. Clin. Invest. **118**:1532.

Mouse CCL2/JE/MCP-1 Polyclonal Antibody
(Catalog # AF-479-NA)
Sample: Rat spinal cord
Application: Immunohistochemistry

Total Nitric Oxide and Nitrite/Nitrate Parameter™ Assay Kit
(Catalog # KGE001)
Sample: Mouse EOC 20 microglial cell line supernates
Application: Colorimetric biochemical assay

9. Christophi, G.P. *et al.* (2008) SHP-1 deficiency and increased inflammatory gene expression in PBMCs of multiple sclerosis patients. Lab. Invest. **88**:243.

Recombinant Human IL-2
(Catalog # 202-IL)
Sample: Human peripheral blood mononuclear cells
Application: Bioassay- proliferation

Recombinant Human IL-4
(Catalog # 204-IL)
Sample: Human peripheral blood mononuclear cells
Application: Bioassay- signaling

Human ADAM8 Ectodomain Phycoerythrin-labeled Monoclonal Antibody
(Catalog # IC10311P)
Sample: Human peripheral blood mononuclear cells
Application: Flow cytometry

Human TNF-β/TNFSF1B Phycoerythrin-labeled Monoclonal Antibody
(Catalog # IC2111P)
Sample: Human peripheral blood mononuclear cells
Application: Flow cytometry

Human CCL17/TARC Phycoerythrin-labeled Monoclonal Antibody
(Catalog # IC3641P)
Sample: Human peripheral blood mononuclear cells
Application: Flow cytometry

10. Xu, J. *et al.* (2007) Peroxisome proliferator-activated receptor-delta agonist fenofibrate regulates IL-12 family cytokine expression in the CNS: relevance to multiple sclerosis. J. Neurochem. **103**:1801.

Mouse IL-27 p28 Quantikine ELISA Kit
(Catalog # M2728)
Sample: Mouse astrocytes and microglial cell supernates
Application: ELISA

R&D SYSTEMS REPRESENTATIVES WILL BE AVAILABLE AT THE FOLLOWING CONFERENCES:

Society for Neuroscience BOOTH # 2409
Washington, D.C. . . . . November 15-19, 2008

Society for Glycobiology BOOTH # 7
Ft. Worth, TX . . . . . November 12-15, 2008

Autumn Immunology
Chicago, IL . . . . . November 21-24, 2008

American Society of Hematology BOOTH # 1754
San Francisco, CA . . . . . December 6-9, 2008

American Society for Cell Biology BOOTH # 1501
San Francisco, CA . . . . . December 13-17, 2008

NEW TOOLS: R&D Systems Neural Toxicity Assay Kit

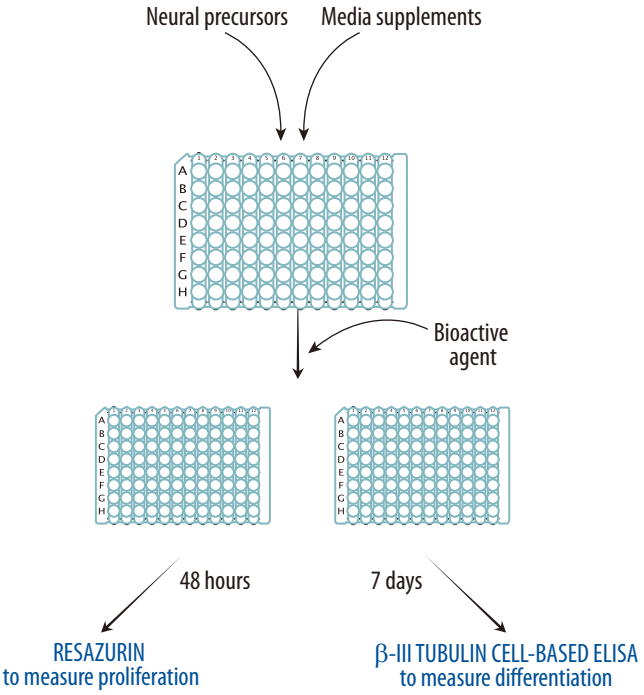
96-Well Format to Screen for Effects of Bioactive Agents on Differentiation & Proliferation

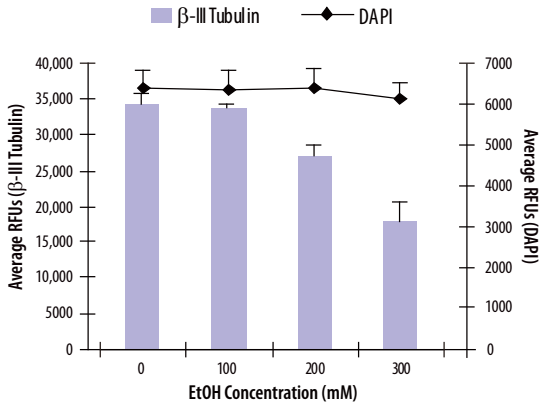
R&D Systems is pleased to offer a new screening tool to identify modulators of neural progenitor cell proliferation and differentiation. The 96-well format provides an easy method to screen for toxins, drugs, growth factors, and other substances that may affect neural stem cells. The Neural Toxicity Assay Kit (Catalog # SC014) can be used to determine the effects on both proliferation and differentiation; either adverse effects as with a toxin, or increased rates of proliferation and differentiation resulting from a stimulatory agent.

Kit Components

- Supplements for maintenance and differentiation
- Fibronectin for plate coating
- Resazurin to monitor proliferation
- β-III tubulin ELISA to monitor differentiation
- Detailed protocols & sample data

Assay Principle





Effect of Ethanol (EtOH) on neuronal differentiation of neural progenitor cells. EtOH has been shown to reduce levels of neuronal differentiation of neural progenitor cells. Rat cortical stem cells (Catalog # NSC001) were differentiated in the presence of the indicated amounts of EtOH. Levels of neuronal differentiation were determined using the Neural Toxicity Assay Kit (Catalog # SC014) as assessed by β-III Tubulin levels. Cells were stained with DAPI as a relative measure of total cell number. Constant levels of DAPI staining indicate that decreases in β-III Tubulin levels are the result of reduced differentiation rather than cell death.

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