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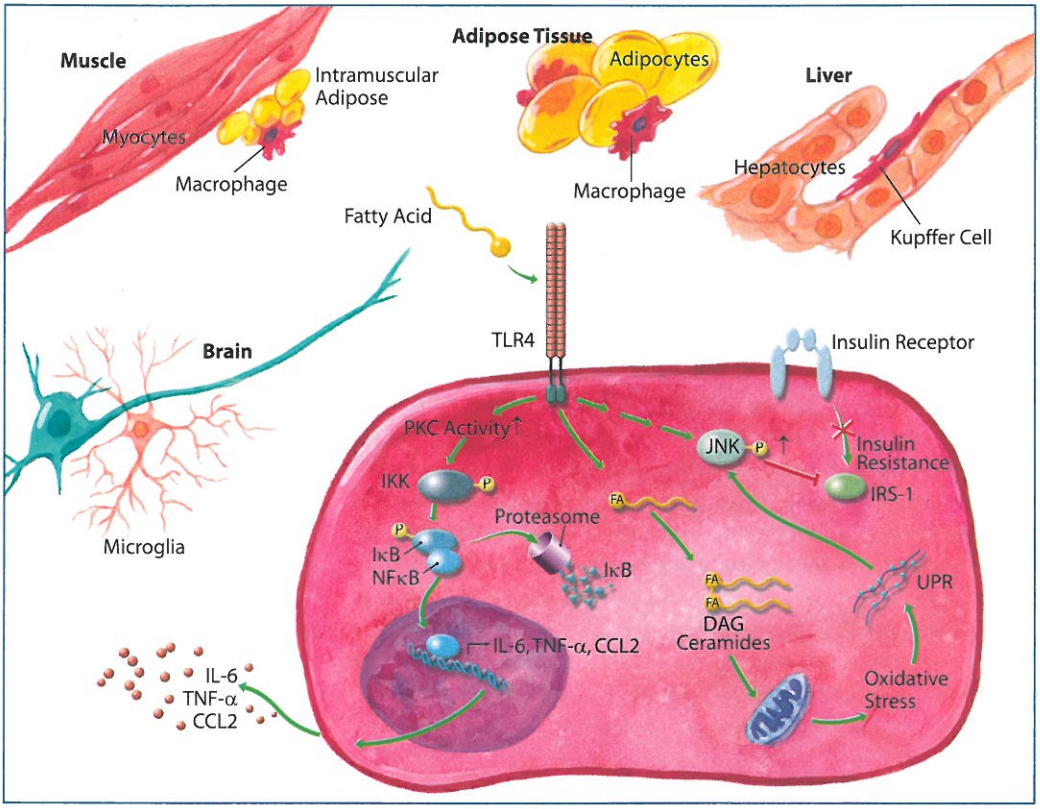

TLR4 Activation by Fatty Acids Triggers a Cellular Response that Promotes Insulin Resistance. In adipose, muscle, brain, and liver tissue, activation of TLR4 by long-chain saturated fatty acids (FA) in either macrophage-lineage cells or resident cells initiates signaling that influences cellular metabolism. An increase in PKC activity leads to the phosphorylation and activation of IKK. IKK phosphorylates IκB, resulting in the nuclear translocation of NFκB, which promotes the transcription of inflammatory cytokines. Formation of diacylglycerols (DAG) and ceramides from internalized fatty acids leads to oxidative stress and the unfolded protein response (UPR). In addition, TLR4 activation stimulates the phosphorylation and activation of JNK, resulting in inhibitory phosphorylation of IRS-1 and insulin resistance.

TLR4: Contributing to Metabolic Syndrome in Multiple Tissues

Obesity has increased dramatically in the United States with serious health consequences.[1] The metabolic consequences of obesity, often termed metabolic syndrome, include lipotoxicity, inflammation, and insulin resistance, which increase the risk of type II diabetes, atherosclerosis, hypertension, and cardiovascular disease. Obesity-related lipotoxicity is thought to result from overloading the body's mechanisms for dealing with dietary lipids. Under normal physiological conditions, adipocytes efficiently convert dietary fatty acids to triglycerides, which can be safely stored in fat droplets. Excess lipids in the form of diacylglycerides and ceramides create stress within the cell, while excess free fatty acids enter the circulation. Adipose tissue hypoxia likely increases oxidative stress on the endoplasmic reticulum, contributing to the unfolded protein response.[1] Kinases including IKK (IκB Kinase) and JNK (c-Jun N terminal Kinase) are activated, leading to the secretion of inflammatory cytokines and inhibitory phosphorylation of IRS-1 (Insulin Receptor Substrate 1), which promotes insulin resistance.[1] During the investigation of these signaling pathways, it has become increasingly apparent that the toll-like receptors (TLRs), and in particular TLR4, may be involved in their initiation.[1,2]

TLRs are mainstays of the innate immune system. Mammalian TLRs include intracellular and extracellular receptors that recognize microbial proteins, nucleic acids, carbohydrates, and lipids to activate host defense mechanisms. TLR4 is a cell surface, transmembrane protein that primarily recognizes bacterial lipopolysaccharides. At sufficiently high concentrations, endogenous lipids can also be recognized by TLR4.[2] TLR4, and to a lesser extent TLR2, are candidates for activating the immune system in response to

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Differentiation Potential of Induced Pluripotent Stem Cells

Somatic cells that have been reprogrammed to a pluripotent state, known as induced pluripotent stem (iPS) cells, are generating excitement due to their ability to function like embryonic stem (ES) cells.[1,2] Unlike ES cells, iPS cells are more readily obtainable for therapy and research, and their isolation does not carry the same ethical concerns.[1,2] Human iPS cells may be an ideal source for patient-specific therapy since they can be derived from the patients themselves. In addition, iPS cells can serve as useful research tools by providing: **1.)** models of human disease to use for screening new drugs or for studying mechanisms of pathogenesis and toxicology, and **2.)** models of normal development to use for screening potential teratogens or for understanding tissue repair and regeneration.[1,3]

iPS cells were first generated by Takahashi and Yamanaka using mouse fibroblasts that had been transduced with four transcription factors: Oct-3/4, SOX2, c-Myc, and KLF4, under ES cell culture conditions.[2] These cells exhibit the morphology, growth, marker expression, and pluripotency of ES cells.[2,3] Further research demonstrated the ability to generate human pluripotent stem cells from adult fibroblasts. Interestingly, there is some flexibility in which transcription factors can be used to reprogram cells. While one report used the same four transcription factors as Takahashi and Yamanaka had used in mice, another study used Oct-4, SOX2, Nanog, and LIN-28 for reprogramming human somatic cells.[4,5] iPS cells appear to exhibit many of the same outward characteristics as ES cells including morphology, proliferation, epigenetic status, and pluripotency.[4,5] However, expression array analysis does suggest that differences in gene expression signatures exist between the cell types.[6] How these differences might affect iPS cells phenotypically is a matter of ongoing research.

Recently, researchers have successfully created mice from iPS cells.[7,8] The success rate for implantation of embryos resulting in viable mice was in the low single digits, and a number of iPS-derived mice exhibited physical abnormalities. Nonetheless, some survived into adulthood and produced viable offspring. In human cells, researchers have demonstrated the capability and efficacy of iPS cells to differentiate into a variety of cell types. Using four different human iPS cell lines, Taura *et al.* were able to show an adipogenic potential comparable to human ES cells.[9] These iPS cells exhibited lipid accumulation and expression of adipogenesis markers including C/EBPα, PPARγ, Leptin, and FABP4. Research from another group showed that iPS cells could be used to generate $CD34^+CD43^+$ hematopoietic progenitors and $CD31^+CD43^-$ endothelial cells under conditions similar to that used for ES cells.[10] These human iPS-derived cells could be further separated into phenotypically defined subsets of primitive hematopoietic cells in a pattern of differentiation resembling that of ES cells. Human iPS cell lines were also induced to differentiate into pancreatic insulin-producing cells.[11] After first generating PDX-1 positive progenitor cells, human iPS cells were further differentiated into pancreatic cells expressing MafA, Glut2, insulin, and in some cases, amylase and C-peptide. Functional cardiomyocytes demonstrating sarcomeric organization and expressing cardiac markers including Nkx2.5, cardiac Troponin T, atrial natriuretic factor, and myosin heavy and light chains, were also derived from human iPS cells and were indistinguishable from those generated from ES cells.[12] Electrophysiology experiments revealed that like ES cells, iPS cells differentiate into nodal-, atrial-, and ventricular-like phenotypes, and are responsive to the canonical cardiomyocyte β-adrenergic signaling pathway. Both human ES and iPS cells were efficiently converted to neural cells using two inhibitors of TGF-β/Smad signaling, Noggin and the drug SB431542.[13] The synergistic action of these two inhibitors resulted in $Pax6^+$ primitive neural cells that could then be further differentiated into neural crest, anterior CNS, somatic motoneurons, and dopaminergic neurons.

These examples of iPS cells differentiating into various somatic cell types pave the way for generating patient-specific pluripotent cells that could be used for research and therapy. Disease research, drug development, and patient therapy will be greatly enhanced by the ability to recapitulate normal and pathologic tissue differentiation and formation *in vitro*, which iPS cells provide more readily than ES cells.

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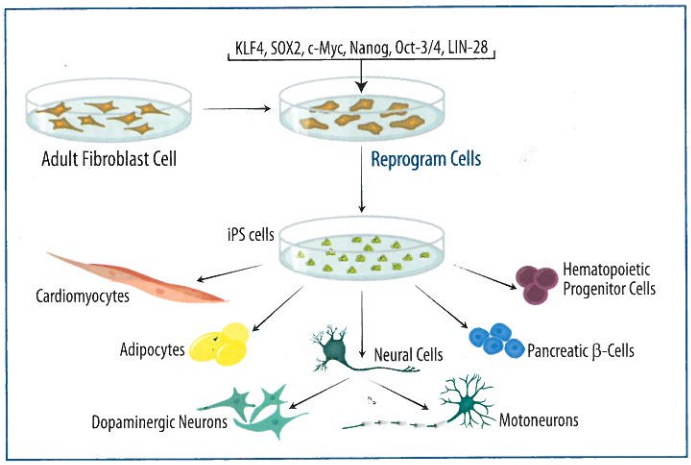
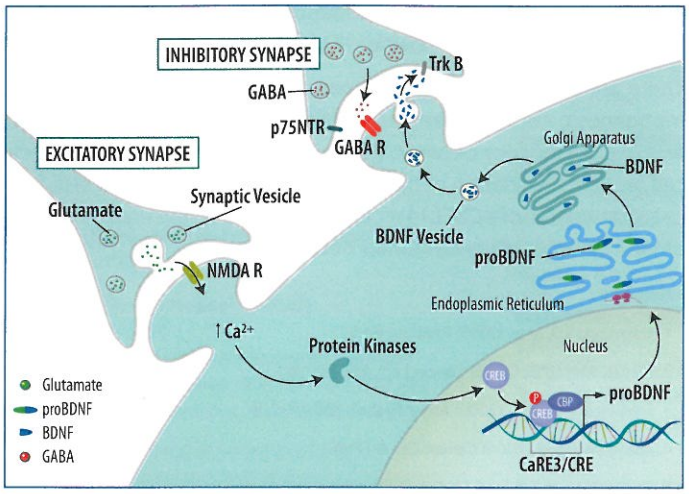


Directed Differentiation of iPS Cells. Induced pluripotent stem cells (iPS cells) are generated by reprogramming adult somatic cells. Following isolation, somatic cells are cultured *in vitro* and transduced with expression vectors encoding transcription factors associated with pluripotency. For most cell types, four factors (c-Myc, Oct-3/4, SOX2, and KLF4) are used, although a combination of alternate factors (Oct-3/4, SOX2, Nanog, and LIN-28) has also been used successfully. Expression of these exogenous factors triggers a gradual process of silencing markers of the differentiated phenotype and inducing markers of the pluripotent state in some cells. As pluripotent cells, iPS cells theoretically have the ability to generate all cell types found in the body. Human iPS cells have been differentiated into a variety of these, including adipocytes, cardiomyocytes, primitive hematopoietic cells, pancreatic β-cells, and several different neuronal cell types. [Figure adapted from Amabile, G. & A. Meissner (2009) Trends Mol. Med. **15**:59.]

Synaptic BDNF: Connecting Physiology to Therapy

Classic effects of brain-derived neurotrophic factor (BDNF) include promoting neuronal viability, differentiation, migration, and dendritic arborization. In addition to these established actions, an evolving body of literature describes an important role at the synapse, where BDNF affects development, function, and plasticity.[1,2] Recently, studies of the synaptic actions of BDNF have focused on activity-dependent changes in neuronal BDNF expression.[3] Activity-dependent changes are those that occur following synaptic transmission, when Ca^{2+} influx induces transcription factors to bind calcium-response elements (CaREs) in the nucleus. Although the importance of activity-regulated BDNF expression for brain development and neuronal function is unquestioned, direct evidence supporting its role in synaptic plasticity has been difficult to obtain.



Activity-dependent BDNF Expression Influences Homeostatic Plasticity. Excitatory neuronal activity increases postsynaptic BDNF levels via Ca^{2+}-dependent transcription factors. For example, CREB phosphorylated on serine 133 binds to CaRE3/CRE following co-activation by CREB binding protein (CBP). Postsynaptic release of BDNF subsequently promotes the formation of inhibitory GABAergic synapses. The BDNF precursor, proBDNF is also an actively secreted molecule that affects synaptic plasticity during development.[12,13]

To address this challenge, Hong *et al.* conducted a series of genetic studies in which individual CaREs in promoter IV of the *Bdnf* gene were blocked using knock-in mutations.[4] Promoter IV is known to be important for synaptic plasticity, both developmentally and in the adult brain. One intriguing finding from this report was observed in CREmKI mice, where the CaRE that regulates activity-dependent *Bdnf* mRNA expression via the CREB transcription factor (CaRE3/CRE) was specifically blocked. Unlike mice lacking BDNF, which are not viable, CREmKI mice developed normally, and were behaviorally indistinguishable from their control littermates. However, the levels of activity-induced *Bdnf* mRNA expression were less than 50% of wild-type control values both in embryonic cultures of CREmKI cortical neurons and in adult visual cortex. This specific reduction in activity-induced *Bdnf* expression decreased inhibitory synapse density in both dissociated cultures of CREmKI neurons and *in vivo* (visual cortex). These novel data suggest that BDNF influences homeostatic plasticity by modulating the balance between excitatory and inhibitory signaling in the brain. This hypothesis was supported by a study which showed that disruption of promoter IV-induced *Bdnf* expression specifically impaired inhibitory synaptic signaling in the prefrontal cortex.[5]

Neurodegeneration and synaptic loss characterize many neurological disorders including Alzheimer's disease (AD), the most common form of dementia.[6] To investigate the potential therapeutic benefits of BDNF administration, Nagahara and colleagues designed experiments to deliver BDNF into the brains of rodents and primates.[7] The authors targeted the cortex and hippocampus, two brain regions that are severely affected during AD. Lentiviral vectors constitutively expressing *Bdnf-GFP* were injected into the entorhinal cortex of a transgenic mouse model of AD. BDNF delivery reversed synaptic loss, as measured by the expression of synaptic markers. In parallel, infusion of recombinant BDNF into the entorhinal cortex of cognitively impaired aged rats improved spatial learning and memory performance in the Morris water maze. These positive findings extended to primate studies where BDNF delivery significantly improved visuospatial discrimination in aged impaired monkeys. The therapeutic potential of BDNF may also apply to Huntington's disease (HD),[8] since increased endogenous BDNF expression rescued synaptic plasticity in a mouse model of HD.[9]

Pharmacologically harnessing the beneficial synaptic effects of BDNF will require a better understanding of the underlying mechanism(s) of its action. Dean *et al.* recently described the role of Synaptotagmin-IV (syt-IV) in BDNF release.[10] In this study, the authors used co-cultures of wild-type and *Syt-IV*$^{-/-}$ mouse hippocampal neurons to investigate the mode of synaptic BDNF release. Results from these experiments showed that neuronal activity upregulates the expression of syt-IV, and syt-IV localizes to BDNF-containing vesicles. Functionally, syt-IV was found to negatively regulate the release of BDNF, an effect which was shown to modulate synaptic function and plasticity. Interestingly, these studies suggested that axons and dendrites have distinct modes of BDNF release, and that postsynaptic BDNF release may act as a retrograde signal to modify presynaptic vesicle fusion.[10] In support of this hypothesis, transcellular retrograde signaling by BDNF was shown to increase hippocampal neuron GABA release via presynaptic TrkB receptors.[11]

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Paradoxical Effects of CXCL14 on Tumorigenesis

CXCL14/BRAK is a chemokine associated with tumor development. In noncancerous tissue, CXCL14 is predominantly expressed in the epithelium, but during tumorigenesis it shows an altered expression pattern. This change, in combination with the effects of CXCL14 on tumor cell proliferation, angiogenesis, and immune cell infiltration, may influence the progression of a range of cancers.

In some cancers, such as those of the prostate and pancreas, CXCL14 is upregulated.[1,2] In contrast, CXCL14 expression is frequently suppressed in tumor cells of the head and neck, breast, cervix, and kidneys.[3,4] In cases of reduced tumor cell expression, production of CXCL14 can be maintained or upregulated in neighboring cells. In head and neck, renal cell, and prostate carcinomas for example, CXCL14 is upregulated in local fibroblasts and the surrounding epithelium.[1,5,6] In breast cancer, CXCL14 is preferentially expressed by myoepithelial cells rather than tumor cells.[2] A caveat here is that these studies determined CXCL14 expression by detection of mRNA or intracellular protein, approaches that may not accurately predict the amount of bioavailable CXCL14. This point is underscored by Peterson *et al.* who observed that immortalized cells and cancer cells can retain expression of the CXCL14 message but chemokine secretion is prevented by proteasome-dependent degradation.[7] Interestingly, elevated levels of intracellular reactive oxygen species induce CXCL14 upregulation in breast cancer cells, leading to accumulation in the endoplasmic reticulum (ER). Under these circumstances, CXCL14 directly interacts with the inositol 1,4,5-trisphosphate (IP_3) receptor in the ER to induce calcium release into the cytoplasm.[8]

CXCL14 exerts paradoxical effects on tumor growth. Exogenous CXCL14 increases the motility and invasiveness of breast and pancreatic cancer cells *in vitro*.[4,8] In contrast, overexpression of CXCL14 in tumor cells of xenograft mouse models of oral squamous cell and prostate cancers suppresses the number and size of the resulting tumors.[3,9] To study the function of stromal cells in tumorigenesis, Augsten *et al.* implanted mice with a mixture of fibroblasts and non-tumorigenic prostate cancer cells.[6] Under these conditions, the mice developed tumors, and the use of CXCL14-overexpressing fibroblasts accelerated tumor growth and vascularization. The increased density of tumor vascularization was potentially due to CXCL14-induced upregulation of FGF basic and VEGF-A, -B, and -C in the fibroblasts rather than a direct effect of CXCL14 on the vasculature.[6] In fact, Shellenberger *et al.* showed that CXCL14 inhibits the activity of angiogenic factors on human umbilical vein endothelial cells and human microvascular endothelial cells, possibly through its ability to directly bind to immobilized CXCL8/IL-8 and FGF basic.[5]

Tumor cell-derived CXCL14 promotes an influx of immature dendritic cells *in vivo* and triggers their activation *in vitro*.[10] Downregulation of CXCL14 production in tumors facilitates tumor growth by attracting fewer dendritic cells, thereby limiting the initiation of an anti-tumor immune response.[10] Leukocytes, vascular endothelial cells, and tumor cells are all potential effectors of CXCL14 activity. They express high and low affinity binding sites for CXCL14 that can be blocked by heparin, although the identity of these receptors remains unknown.[2,5]

There are clearly unexplained aspects of the role of CXCL14 in tumorigenesis, notably its inconsistent expression among tumor types and the discrepancy between its *in vitro* and *in vivo* effects on tumor cell growth. Elucidation of the participation of local stromal cells and characterization of a CXCL14 receptor will advance the understanding of how CXCL14 influences tumor development.

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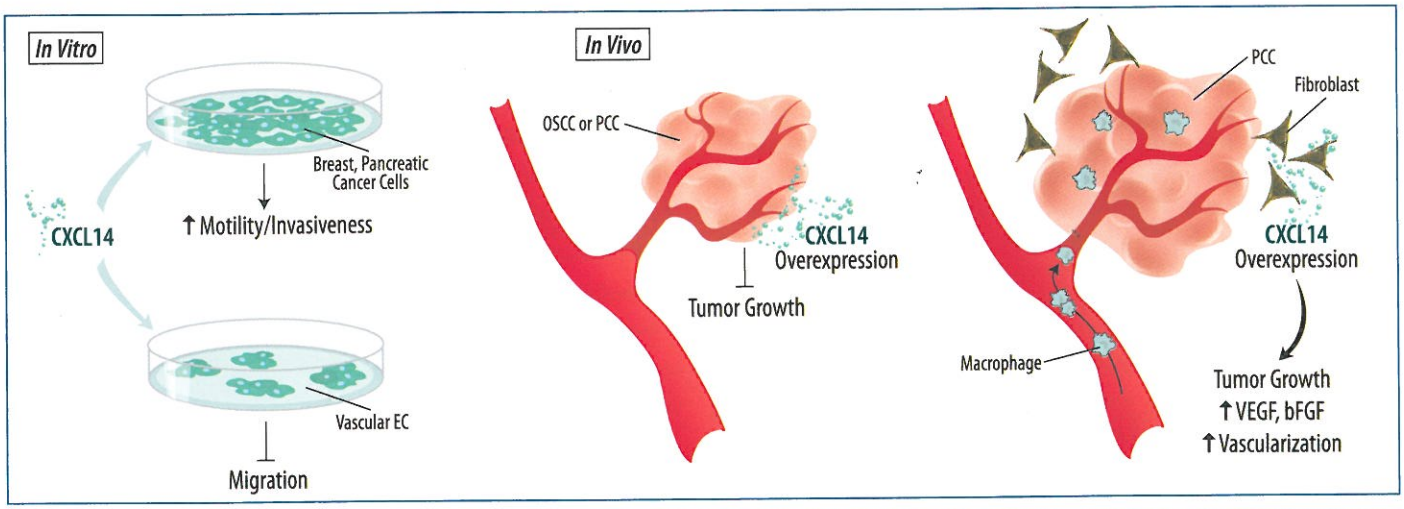

CXCL14 Exerts Paradoxical Effects on Tumorigenesis. CXCL14 exhibits contradictory effects on tumorigenesis under different experimental conditions. *In vitro*, CXCL14 promotes breast and pancreatic tumor cell invasion and inhibits vascular endothelial cell (EC) migration. *In vivo*, overexpression of CXCL14 in oral squamous carcinoma cells (OSCC) and prostate cancer cells (PCC) suppresses tumorigenesis, while overexpression of CXCL14 in prostate cancer-associated fibroblasts induces tumor growth and vascularization. Fibroblast-derived CXCL14 also stimulates monocyte migration *in vitro* suggesting that it may promote macrophage recruitment *in vivo*.

RECENT CITATIONS: R&D Systems Products for Angiogenesis Research

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Recombinant Human Angiopoietin-1
(Catalog # 923-AN)

Recombinant Human Angiopoietin-2
(Catalog # 623-AN)
Sample: Mouse b-END3 brain-derived microvascular endothelial cells, human umbilical vein, and human EA.hy926 endothelial cells
Application: Bioassay- signaling

Recombinant Human Tie-2/Fc Chimera
(Catalog # 313-TI)
Sample: Human umbilical vein endothelial cells
Application: Pull-down and Cell adhesion assays

Recombinant Human VEGF R3/Flt-4/Fc Chimera
(Catalog # 349-F4)

Recombinant Human Tie-1/Fc Chimera
(Catalog # 619-TI)
Sample: Human umbilical vein endothelial cells
Application: Pull-down and Bead aggregation assays

Phospho-Tie-2 (Y992) Polyclonal Antibody
(Catalog # AF2720)

Human Tie-2 Polyclonal Antibody
(Catalog # AF313)
Sample: Human umbilical vein, human pulmonary microvascular, and human EA.hy926 endothelial cells
Applications: Western Blot, Immunohistochemistry

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Human Angiopoietin-1 Quantikine® ELISA Kit
(Catalog # DANG10)

Human Angiopoietin-2 Quantikine ELISA Kit
(Catalog # DANG20)

Human VEGF Quantikine ELISA Kit
(Catalog # DVE00)
Sample: Porcine myocardium homogenate
Application: ELISA

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Human FGF basic Quantikine ELISA Kit
(Catalog # DFB50)
Sample: Human microvascular endothelial cell supernates
Application: ELISA

Human FGF basic Polyclonal Antibody
(Catalog # AF-233-NA)
Sample: Rat aortic ring explant
Application: Neutralization

Recombinant Human FGF basic
(Catalog # 233-FB)
Sample: Rat aortic ring explant
Application: Bioassay- microvessel outgrowth

4. Crawford, Y. *et al.* (2009) PDGF-C mediates the angiogenic and tumorigenic properties of fibroblasts associated with tumors refractory to anti-VEGF treatment. Cancer Cell **15**:21.

Recombinant Human PDGF-DD
(Catalog # 1159-SB)

Recombinant Mouse VEGF 164
(Catalog # 493-MV)

Recombinant Human PDGF-CC
(Catalog # 1687-CC)
Application: Western blot specificity control

Recombinant Human/Rhesus Macaque/Feline CXCL12/SDF-1α
(Catalog # 350-NS)
Sample: Mouse normal skin and tumor-associated fibroblasts
Applications: *In vivo* Matrigel® plug assay, Western blot specificity control

Recombinant Mouse PDGF-CC
(Catalog # 1447-PC)
Sample: Mouse normal skin and tumor-associated fibroblasts and endothelial cells
Applications: *In vivo* Matrigel plug assay, Bioassay-migration, Western blot positive control

Mouse PDGF-C Polyclonal Antibody
(Catalog # AF1447)
Sample: Mouse normal skin and tumor-associated fibroblasts; Recombinant mouse PDGF-CC
Applications: Neutralization of *In vivo* Matrigel plug assay, Western blot

Mouse VEGF Quantikine ELISA Kit
(Catalog # MMV00)
Sample: Mouse normal skin and tumor-associated fibroblast tumor supernates
Application: ELISA

5. Zacchigna, S. *et al.* (2008) Bone marrow cells recruited through the neuropilin-1 receptor promote arterial formation at the sites of adult neoangiogenesis in mice. J. Clin. Invest. **118**:2062.

Rat Neuropilin-1 Polyclonal Antibody
(Catalog # AF566)

Rat Neuropilin-2 Polyclonal Antibody
(Catalog # AF567)
Sample: Mouse muscle
Application: Immunohistochemistry

Recombinant Human VEGF 165
(Catalog # 293-VE)

Recombinant Human VEGF (aa 207-318)
(Catalog # 298-VS)

Recombinant Human Semaphorin 3A/Fc Chimera
(Catalog # 1250-S3)
Sample: Human smooth muscle and endothelial cells
Application: Bioassay- migration

6. Tammela, T. *et al.* (2008) Blocking VEGFR-3 suppresses angiogenic sprouting and vascular network formation. Nature **454**:656.

Mouse VEGF R3/Flt-4 Polyclonal Antibody
(Catalog # AF743)

Mouse VEGF R2/Flk-1 Polyclonal Antibody
(Catalog # AF644)

Mouse DLL4 Polyclonal Antibody
(Catalog # AF1389)
Sample: Mouse retina and skin
Application: Immunohistochemistry

7. Zhang, Z. *et al.* (2008) α2β1 integrin expression in the tumor microenvironment enhances tumor angiogenesis in a tumor cell-specific manner. Blood **111**:1980.

Recombinant Mouse VEGF 164
(Catalog # 493-MV)

Recombinant Human PlGF
(Catalog # 264-PG)
Sample: Mouse and mouse thoracic aortal explant
Application: *In vivo* Matrigel plug assay, Bioassay-aortic ring assay

Recombinant Human VEGF-C
(Catalog # 2179-VC)
Sample: Mouse
Application: *In vivo* Matrigel plug assay

Mouse VEGF R2/Flk-1 Monoclonal Antibody
(Catalog # MAB4431)

Mouse VEGF R1/Flt-1 Polyclonal Antibody
(Catalog # AF471)
Sample: Tumor, pulmonary endothelial cells, and lysates
Application: Immunohistochemistry, Neutralization, Western blot

Mouse PlGF-2 Quantikine ELISA Kit
(Catalog # MP200)

Mouse VEGF Quantikine ELISA Kit
(Catalog # MMV00)
Sample: Mouse Lewis lung carcinoma and B16-F10 melanoma cell line supernates
Application: ELISA

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Recombinant Human MMP-14
(Catalog # 918-MP)
Sample: Human Fab displaying phage library
Application: Library screening and selection

Human VEGF Monoclonal Antibody
(Catalog # MAB293)
Sample: Human umbilical vein endothelial cells
Application: Neutralization

Human MMP-14 Monoclonal Antibody
(Catalog # MAB9181)
Sample: Human umbilical vein endothelial, HT-1080 fibrosarcoma, MDA-MB-231, and MCF-7 breast cancer cell lysates
Application: Western Blot

TECHNICAL NOTE: R&D Systems
Structural Characterization of the 1918 Influenza Virus Neuraminidase

The 1918 viral influenza pandemic was one of the deadliest flu outbreaks in history with approximately 20-50 million deaths worldwide.[1] Due to the emergence of the 2009 H1N1 influenza pandemic and the likelihood of future flu pandemics, the molecular characteristics accounting for the virulence of the 1918 virus are of great interest. Two viral envelope glycoproteins, Hemagglutinin (HA) and Neuraminidase (NA), have been the focus of numerous studies due to their abilities to affect the viral infectious cycle and host specificity. While the HA protein mediates viral attachment to the host cell membrane, the NA protein removes sialic acid on host cells and viral particles to promote viral release and prevent self-aggregation. The importance of the NA protein to the infectivity of the 1918 virus was demonstrated in a study in which the NA gene in the 1918 virus was replaced with that from a contemporary, less virulent H1N1 virus, Tx/91.[1] Five days after inoculation, replacement of the NA gene reduced the infectivity titer at least 100-fold compared to the wild-type virus.

Scientists at R&D Systems have now identified structural features of the 1918 viral NA protein that may have contributed to the robust infectivity of the virus.[2] The protein was purified from baculovirus-expressing *Sf*21 insect cells and separated by gel filtration. It appeared as a monomer, dimer, and tetramer, but only the tetramer retained enzymatic activity. Significantly, the monomer and dimer could not be oligomerized into the tetramer in solution, suggesting that unique structural elements are required for NA oligomerization and activation. Differences in the molecular masses of the monomer and tetramer under reducing conditions led to an analysis of their N-glycosylation patterns. While treatment of the tetramer with a series of endoglycosidases revealed a different N-glycan profile than the monomer (Figure 1), N-deglycosylation reduced, but did not abolish its enzymatic activity (Figure 2). The observed decrease in activity was largely due to destabilization of the tetramer, indicating that glycosylation is required for proper folding of the NA protein. In addition, the NA tetramer was found to be resistant to trypsin digestion in contrast to both the monomer and dimer (Figure 3). Further analysis revealed that the stalk region of the NA protein, a site which is typically vulnerable to host protease attack in other influenza viruses, contains five N-glycosylation sites and no trypsin cleavage sites in the 1918 viral NA. Both of these features likely protect the NA protein from cleavage by host proteases. As a point of comparison, NA from the less virulent Tx/91 virus contains two different N-glycosylation sites and three tryptic sites in its stalk region.[2] These findings led to the speculation that a unique glycosylation pattern and trypsin resistance in the stalk of the NA protein may have allowed the 1918 virus to more robustly infect a wider range of tissues.

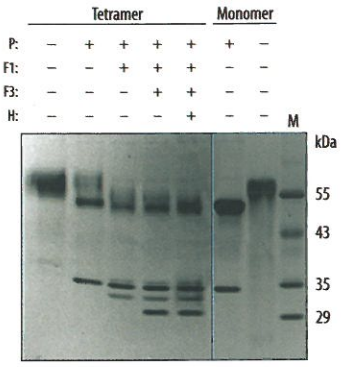

Figure 1. The Active NA Tetramer has a Different N-glycan Profile than the Inactive Monomer. The NA tetramer was treated with different combinations of endoglycosidases under native conditions for 20 hours and then separated on a reducing SDS gel. While the monomer was only deglycosylated by PNGase F (P) and Endoglucosaminidase F3 (not shown), the tetramer was partially deglycosylated by all four endoglycosidases revealing a distinct N-glycan profile. P, PNGase F; F1, Endoglucosaminidase F1; F3, Endoglucosaminidase F3; and H, Endoglucosaminidase H. M = Molecular weight marker.

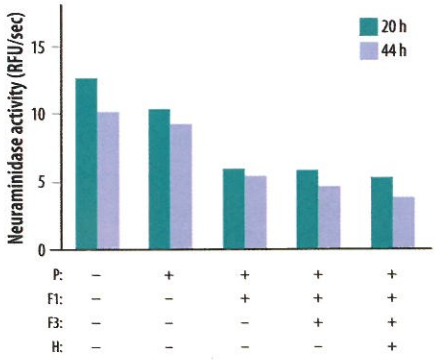

Figure 2. Deglycosylation of the NA Tetramer Reduces but does not Abolish Its Enzymatic Activity. The enzymatic activity of the NA tetramer was measured after 20 hours (aqua bars) and 44 hours (purple bars) of treatment with different combinations of endoglycosidases. P, PNGase F; F1, Endoglucosaminidase F1; F3, Endoglucosaminidase F3; and H, Endoglucosaminidase H.

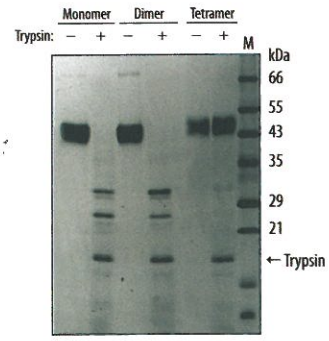

Figure 3. The NA Tetramer is Resistant to Trypsin Digestion. The NA monomer, dimer, and tetramer were either untreated (-) or treated (+) with trypsin and then separated by SDS-PAGE. Proteins were visualized by staining the gel. Trypsin is indicated by the arrow. M = Molecular weight marker.

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TLR4 continued from page 1

a diet rich in long-chain saturated fatty acids. In 2007, a pivotal study demonstrated that mice with a loss-of-function mutation in TLR4 resist becoming obese on a high-fat diet.[3] This places TLR4 among several other proteins whose inactivation can protect against high fat diet-induced insulin resistance.

TLR4 is expressed on macrophages, which are recruited by inflammatory cytokines in tissues such as obese adipose tissue. In addition, it is also expressed on resident, nonimmune cells, such as adipocytes and muscle myocytes.[4,5] Muscle accounts for most insulin-stimulated glucose use, and is therefore a key insulin target tissue. Treatment of isolated muscle with long-chain saturated fatty acids such as palmitate activates both JNK- and IKK/NFκB-mediated pathways, promotes insulin resistance, and enhances cellular output of inflammatory cytokines such as IL-6, CCL2/JE/MCP-1, and TNF-α.[3,6] In contrast, muscle deficient in TLR4 activity lacks these effects.[3,6] In humans, recent studies show that TLR4 expression is upregulated in muscle or adipose tissue from obese or type II diabetic subjects, and this increase correlates with insulin resistance.[7,8] In addition, there is an increase in TLR4-mediated NFκB activation. Interestingly, mono-unsaturated fatty acids such as olein, which is enriched in olive oil, appear to neutralize the effects of palmitate and thus have a positive rather than a negative effect.[9] Exercise also has a positive effect, downregulating the expression of TLR4 and decreasing palmitate accumulation in human muscle.[10]

New data also links activation of TLR4, and possibly TLR2, on macrophages or their tissue-specific relatives, microglia and Kupffer cells, to obesity-related events in their respective tissues. In the brain, TLR engagement increases cytokine expression, stress in the endoplasmic reticulum, and leptin resistance. These effects stimulate systemic inflammation, which promotes atherosclerosis in blood vessels and contributes to non-alcoholic fatty liver disease.[1,11-13] While far from being the only cell surface molecule involved, TLR4 does seem to link many known signaling pathways involved in the metabolic consequences of obesity.

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