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R&D Systems Cytokine Bulletin
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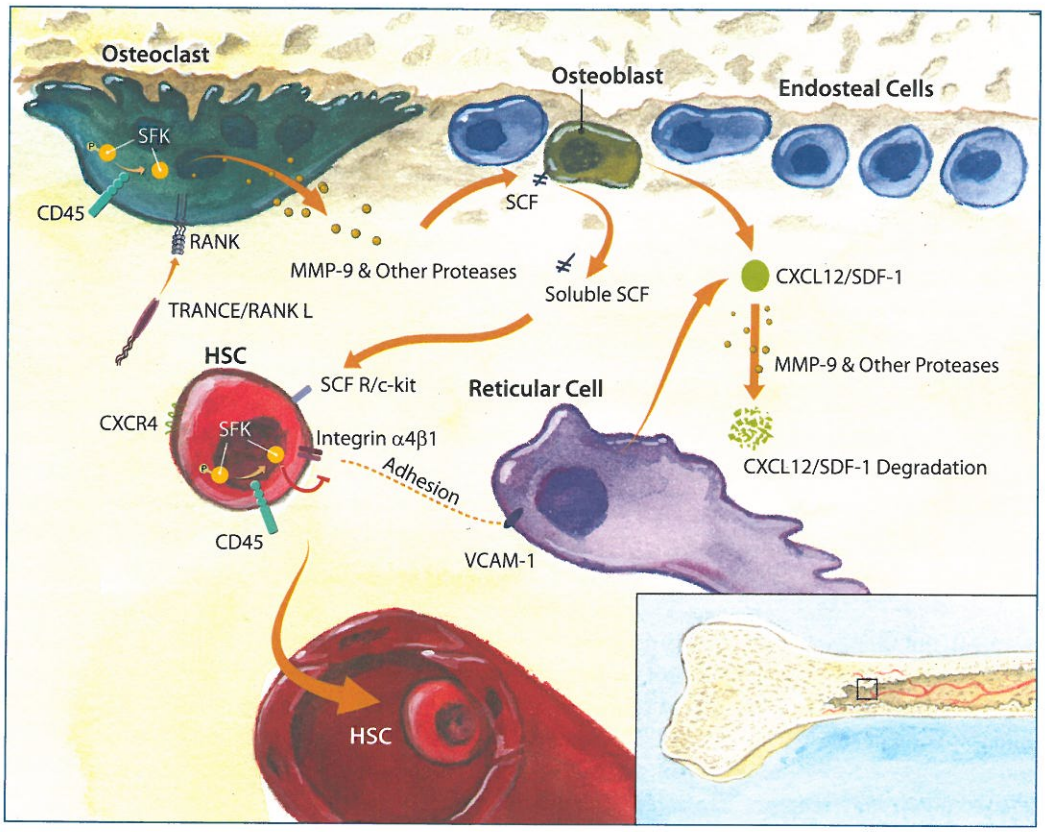


Processes downstream of CD45 are involved in HSC mobilization. CD45 dephosphorylates and downregulates the activity of Src family kinases (SFK) on hematopoietic stem cells (HSCs). This decreases integrin activity and adhesion as occurs, for example, between HSC Integrin α4β1 and reticular cell VCAM-1. CD45 also downregulates osteoclast SFK activity, which allows TRANCE/RANK L to induce the secretion of MMP-9 and other proteases. These proteases catalyze the release of soluble Stem Cell Factor (SCF) from osteoblasts and the degradation of CXCL12/SDF-1, which normally interact with HSC SCF R/c-kit and CXCR4, respectively. Loss of these interactions results in HSC mobilization from the bone marrow into the blood.

CD45 in the Stem Cell Niche

The hematopoietic stem cell (HSC) niche is a complex milieu that supports both the survival of quiescent HSCs, and allows for the expansion, differentiation, and migration of precursors during the process of hematopoietic cell replenishment.[1,2] Stromal cells that support the most primitive HSCs in the endosteal niche are actually a mixture of fibroblasts, CXCL12/SDF-1-expressing reticular cells, endothelial cells, and bone-forming osteoblasts.[1,2] Bone-resorbing osteoclasts have recently been suggested to play a role in the niche as well.[3] Current evidence indicates that this role involves the hematopoietic transmembrane tyrosine phosphatase, CD45.[4]

CD45 is present on the surface of all hematopoietic cells, including HSCs and osteoclasts, which are of hematopoietic origin.[4,5] Src family kinases (SFK) are the most well-studied of its substrates. CD45 can affect SFK activity either negatively or positively, depending on which phosphotyrosine it dephosphorylates.[5] Deletion mutations within CD45 in humans are associated with severe immunodeficiency. This is primarily due to the absence of CD45 on T cells, where it is typically abundant and required to modulate SFK activity during antigen responses.[5] CD45-deficient ($CD45^{-/-}$) mouse bone marrow contains normal numbers of hematopoietic cells, but the most primitive HSCs are reduced in number, and their mobilization in response to G-CSF is impaired.[4] In part, this defect is intrinsic to the HSC; without CD45-mediated downregulation of SFK activity, integrin-mediated adhesion is high and HSCs are more likely to remain in the niche. $CD45^{-/-}$ HSCs are also deficient in G-CSF-stimulated mobilization and homing to the chemokine CXCL12/SDF-1, which negatively affects cell engraftment following transplantation.[4] These deficiencies can be restored by supplementation with SFK inhibitors, indicating that this role is usually performed by CD45.[4] Likewise, $CD45^{-/-}$ recipients also show deficient engraftment and

Continued on page 7

Nerve Growth Factor Receptors & Asthma

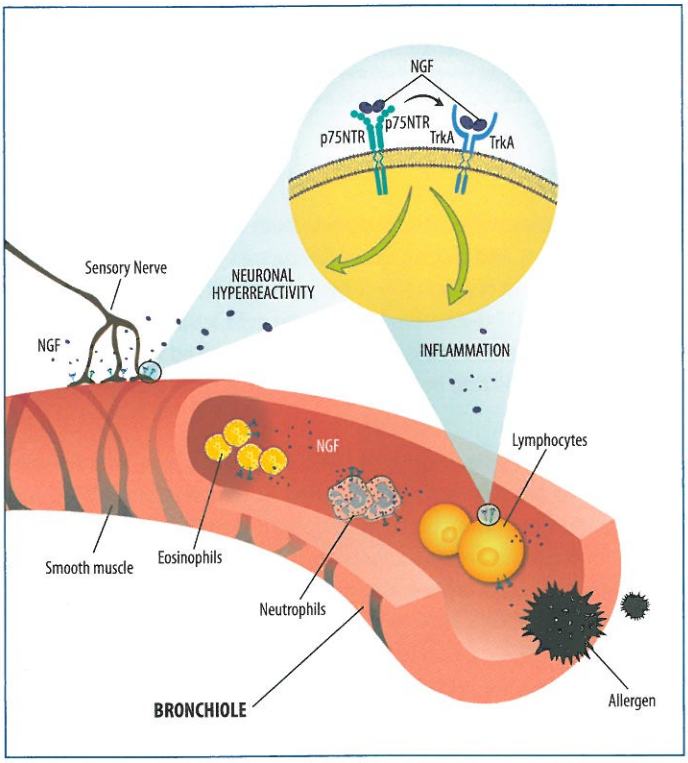
Allergic asthma is an increasingly common disease that is characterized by inflammation of the bronchioles and hypersensitivity of airway smooth muscle and sensory nerves. Allergen inhalation triggers the activation of inflammatory immune cells including eosinophils, mast cells, neutrophils, and T cells, which infiltrate the airway.[1] Local sensory nerve C-fibers release neuropeptides that induce smooth muscle contraction and increase airway sensitivity to a wide range of irritants including cold air and cigarette smoke.[2]

Nerve growth factor (NGF) has been implicated in both the immune and neuronal components of allergic asthma pathogenesis. It is constitutively produced by bronchiolar epithelial and smooth muscle cells, and is upregulated by these cells and infiltrating immune cells during asthma attacks.[3] NGF enhances the allergen-dependent activation, infiltration, and viability of eosinophils, an effect which is reduced by neurotrophin blockade.[4,5] In the normal lung, the receptors for NGF [NGF R/p75 Neurotrophin Receptor (NTR) and TrkA] are restricted to sensory nerve bundles and terminals. Following sensitization and allergen challenge, they are additionally present on infiltrating hematopoietic cells.[5-8] Experimental blockade of the Trk receptors limits sensory nerve hyperreactivity and airway inflammation.[6]

Kerzel *et al.* examined the involvement of NGF R/p75NTR in allergic asthma with the use of knockout mice lacking this receptor.[7] These animals were capable of mounting a systemic allergen-specific IgE response, but they exhibited significantly lower eosinophil counts in the bronchoalveolar lavage fluid (BALF). This reduced eosinophil infiltration was partially mimicked in wild-type mice by intranasal administration of anti-NGF R antibodies. Knockout of NGF R/p75NTR had no effect on hyperresponsiveness to methacholine, an agonist of muscarinic M3 receptors expressed on smooth muscle cells. However, knockout of NGF R/p75NTR caused a reduction in typical hyperresponsiveness to capsaicin, an agonist of TRPV1 vanilloid receptors on C-fiber sensory nerves. As with cellular infiltration, anti-NGF R antibodies partially prevented capsaicin-induced hyperresponsiveness in wild-type mice. The authors concluded that both neuronal hyperreactivity and inflammation require NGF R/p75NTR expression.

To evaluate the relative contribution of NGF R/p75NTR on sensory nerves and hematopoietic cells, Nassenstein *et al.* designed NGF R/p75NTR knockouts in which the receptor is absent on one or both of these cell types.[8] Sensitization and allergen challenge of the mice triggered neuronal hyperreactivity only if NGF R/p75NTR was expressed on sensory nerves, while its presence on immune cells was not involved. In contrast, allergen-provoked eosinophil, lymphocyte, and neutrophil infiltration was dependent on NGF R/p75NTR expression on immune cells, but not on sensory nerves. Therefore the effects of NGF binding to NGF R/p75NTR are determined by which cell type expresses the receptor. The reduced neuronal and immune responses in NGF R/p75NTR knockout mice are due to the absence of the receptor rather than a loss of neurotrophin production, as demonstrated by the unchanged levels of NGF and BDNF in the BALF of mice lacking NGF R/p75NTR in either tissue compartment.

NGF plays an important role in the early stages of allergic asthma by exerting tissue-specific effects on the local nervous and immune systems. Following the onset of an allergic asthma reaction, sensitized nerves reinforce existing airway inflammation by releasing neuropeptides, while activated immune cell-derived mediators aggravate neuronal sensitivity. Modulation of NGF interactions with its receptors shows potential for the control of both of these aspects of allergic asthma.



The Role of Nerve Growth Factor (NGF) in Asthma. During an allergic asthma attack, increased amounts of NGF are produced by bronchiolar smooth muscle cells, endothelial cells, and infiltrating immune cells. The interaction of NGF with NGF Receptors (NGF R/p75 NTR; TrkA) on sensory neurons amplifies neuronal sensitivity, while the interaction of NGF with its receptors on hematopoietic cells promotes cellular activation and infiltration of the bronchiole.

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GDNF: A Novel Treatment for Parkinson's Disease?

Parkinson's disease (PD), a degenerative disorder of the central nervous system, affects nearly 1% of the global population aged 65 and older.[1-2] Primary symptoms of PD include muscle rigidity, tremor, and bradykinesia.[1] As the disease progresses, individuals with PD experience postural instability, cognitive dysfunction (dementia and psychosis), sleep abnormalities, and mood disorders.[1-2] The pathophysiology of the disease stems from the loss of pigmented dopaminergic (DA) neurons in the substantia nigra (SN).[1] These neurons project to the striatum, and their loss leads to alterations in the activity of the neural circuits within the basal ganglia that regulate movement.[1]

Of the pharmacological treatments currently available, none prevent the progressive loss of DA neurons observed during the course of PD.[3] For several years, trophic factors have been pursued as potential therapeutic agents due to their ability to regulate the survival of specific neuronal populations in the central nervous system.[3] One such trophic factor is glial cell-derived neurotrophic factor (GDNF), a member of the transforming growth factor β superfamily. GDNF was first identified on the basis of its ability to support the development of embryonic DA neurons.[4] It has since been shown to protect and restore mature DA neurons in the SN in many different lesion models. The challenge in assessing its therapeutic potential has been finding a way to deliver GDNF to PD patients.

One strategy for delivering GDNF to target tissues that has been explored in animal models of PD is *in vivo* gene transfer using viral vectors. Bensadoun *et al.* unilaterally injected lentiviral vectors carrying GDNF or inactivated GDNF above the SN in mice.[5] Two weeks later, the animals were lesioned on the same side of the striatum with the neurotoxin, 6-hydroxydopamine (6-OHDA). Apomorphine-induced rotational behavior (i.e. circling), indicative of DA neuron loss, was significantly decreased in the GDNF-injected group compared to control animals. GDNF also protected 70% of the tyrosine hydroxylase-positive cells in the SN against 6-OHDA-induced toxicity, compared to 33% in control animals injected with mutated, inactive GDNF. These findings were supported by Kordower *et al.*, who used lentiviral vectors to show that GDNF could prevent nigrostriatal degeneration and reverse functional defects in monkeys treated with the neurotoxin, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP).[6] Adenoviral infection has also been shown to be an effective means of delivering GDNF in animal models of PD. Choi-Lundberg *et al.* demonstrated that 6-OHDA-induced degeneration of DA neurons could be reduced by injecting the SN of rats with a replication-defective adenovirus expressing human GDNF.[7] Six weeks after treatment with 6-OHDA, the loss of DA neurons was reduced approximately threefold in GDNF-infected rats, relative to control rats. Similarly, adenoviral expression of GDNF in mice prevented the depletion of striatal dopamine and the loss of synaptic plasticity associated with MPTP-induced damage.[8]

Other methods of GDNF delivery or induced upregulation have also shown promise in animal models of PD. GDNF-releasing, biodegradable microspheres implanted into the striatum of PD model rats were shown to induce DA fiber sprouting and synaptogenesis.[9] In addition, GDNF expression was shown to be stimulated in MPTP-treated mice both by adoptive transfer of copolymer-1 (Cop-1) immune cells and by striatal transplantation of human amniotic cells.[10,11] A similar increase in GDNF production was observed in MPTP-treated monkeys following intrastriatal or intranigral injection of transgenic human neural progenitor cells, providing hope that one of these strategies may be effective in humans.[12] Recently, advancements have also been reported in the development of therapeutic approaches in monkeys utilizing GDNF-mediated transplantation of fetal DA neurons.[13] These results suggest that GDNF gene therapy combined with neural transplantation may improve the modest gains that have been made thus far in clinical studies of neural grafting.[13]

The neuroprotective and neurorestorative effects of GDNF in MPTP- or 6-OHDA-induced animal models of PD imply that GDNF may be useful for treating PD patients. Unfortunately, GDNF delivery in humans by intracerebroventricular injection or intrastriatal infusion has proven ineffective and other methods of GDNF delivery have yet to be tested.[14] Thus, finding a safe and effective approach to exploit the neuroprotective effects of GDNF remains an active area of research in developing a treatment to inhibit the progression of PD.

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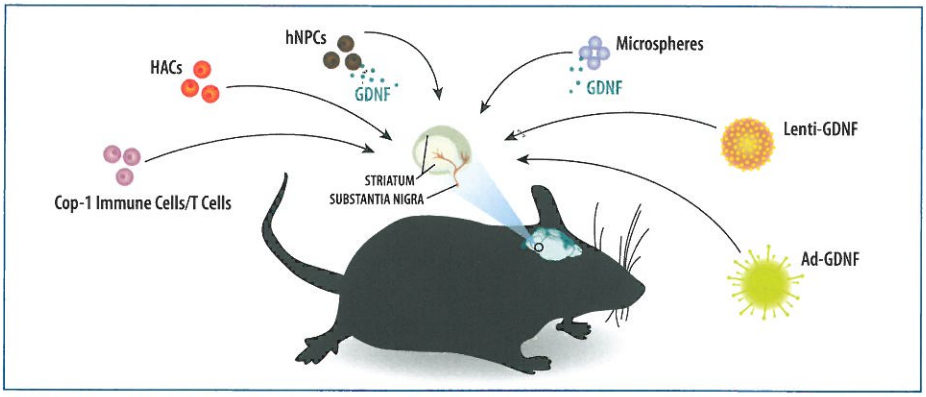

GDNF Delivery Methods Investigated in Animal Models of Parkinson's Disease (PD). Several methods of GDNF delivery have proven to be effective in preventing the loss of dopaminergic neurons in neurotoxin-induced animal models of PD. Ad-GDNF: Adenoviral-expressed GDNF; Lenti-GDNF: Lentiviral-expressed GDNF; hNPCs: Human Neural Progenitor Cells; Cop-1: Copolymer-1 immune cell transfer; HACs: Human Amniotic Cells.

Mouse Ribs & Fly Veins: CV-2 Regulation of BMP Morphogenetic Fields

Originally identified as protein regulators of cartilage and bone formation, bone morphogenetic proteins (BMPs) elicit additional functions during embryogenesis and morphogenesis of various tissues and organs. BMPs regulate growth, differentiation, patterning, chemotaxis, and apoptosis of various cell types and tissues, including mesenchymal, epithelial, hematopoietic, and neuronal cells. The spatial distribution and amount of BMP signaling are tightly regulated by a diversity of extracellular inhibitors, including Noggin, Chordin, DAN family members, and Crossveinless-2. However, extracellular regulation of BMP signaling has proven to be more complex than individual, specific inhibitors binding to BMP and inhibiting its ability to interact with its receptors.

Crossveinless-2 (CV-2), also known as bone morphogenetic protein-binding endothelial cell precursor-derived regulator (BMPER), is a secreted protein containing five tandem chordin-like cysteine-rich domains and a partial von Willebrand factor type D domain.[1,2] Reports show that CV-2 directly interacts with BMP-2, -4, and -6 to antagonize BMP signaling, and CV-2 can block BMP signaling in both animal cap assays and in differentiating embryonic stem cells.[2] In addition, human CV-2 can inhibit BMP-2- and BMP-4-dependent osteoblast differentiation and BMP-dependent differentiation of chondrogenic cells.[1] Paradoxically, genetic data from *Drosophila*, biochemical experiments in COS7 cells, and analysis of organ phenotypes in CV-2 null mice suggest that CV-2 can also potentiate BMP signaling.[3-5] Recently, these disparate findings were reconciled when the mechanism by which CV-2 functions was further described and refined.

During development of the *Drosophila* wing, a BMP gradient modulated by the short-range molecule CV-2, is required for proper crossvein formation.[3] Using a combination of biochemical and genetic data, Serpe *et al.* showed that low CV-2 levels help deliver BMP to its receptor, while high CV-2 levels sequester BMP and antagonize signaling.[6] This biphasic activity, along with positive feedback regulation of CV-2 by BMP signaling, results in sharp boundaries and gives rise to localized and distinct crossveins in the fly wing.[6]

In vertebrates, the impact of CV-2 on BMP signaling is also biphasic, although the molecular mechanism differs. Ambrosio *et al.* demonstrated that CV-2 interacts with Chordin to coordinate diffusion of BMP on the ventral side of the *Xenopus* embryo.[7] When Chordin levels are low, CV-2 cooperates with Chordin to sequester BMP from its receptors.[7] As Chordin levels increase, CV-2 switches from antagonizing BMP to antagonizing Chordin, thereby enhancing BMP signaling.[7]

Further investigation in CV-2 knockout mice revealed an interesting morphogenetic phenotype in the thoracic vertebrae. In mouse embryos, CV-2 is expressed in future vertebral bodies, while BMP-4, Chordin, and Chordin-like proteins are all expressed in the intervertebral disc region. In CV-2 null embryos, the vertebral bodies are reduced in size and the 13th thoracic vertebra displays a posterior homeotic transformation characterized by loss of the 13th rib.[8] By looking at BMP signaling via Smad phosphorylation in this region, Zakin *et al.* determined that CV-2 is required to facilitate BMP signaling in the region adjacent to, but not within, the site of high CV-2 expression.[8] Thus, it appears that CV-2 may help establish morphogenetic fields of BMP signaling resulting in discrete boundaries in mammalian vertebrae in much the same way as it does in the crossveins of insect wings.

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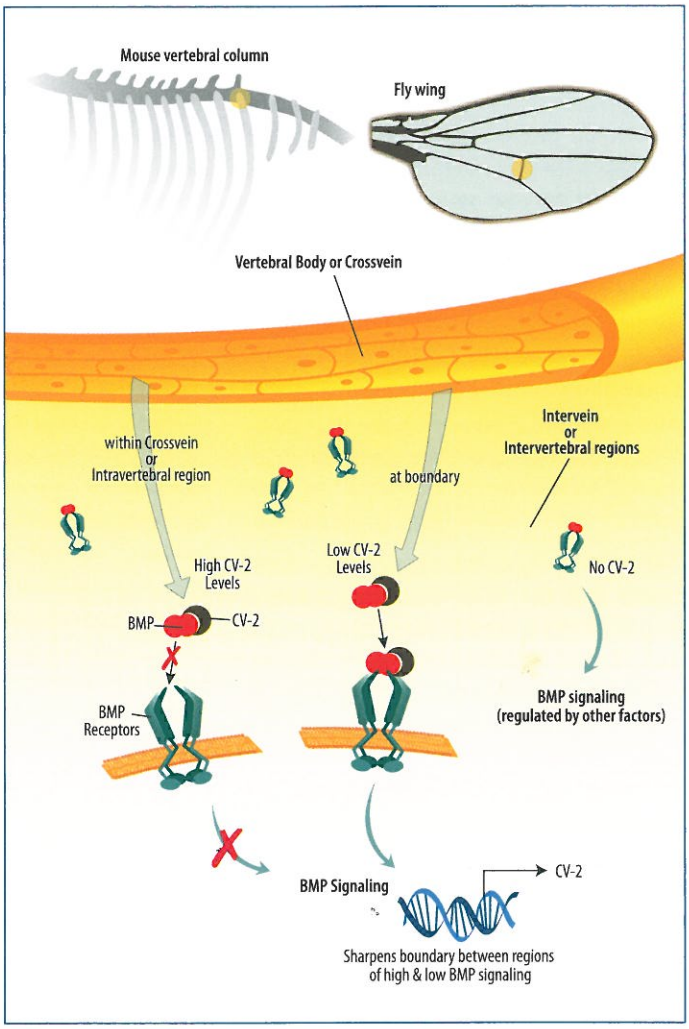

CV-2 regulates BMP signaling to pattern vertebral bodies in the spinal cord and crossveins in the insect wing. High CV-2 levels within vetebral bodies in the spinal cord or crossveins in the fly wing prevent BMP signaling, while low levels at the boundaries promote signaling. BMP signaling activates transcription of CV-2 and this positive feedback helps sharpen the boundary between regions of high and low CV-2 expression.

RECENT CITATIONS:
R&D Systems Products for Bone Development & Disease Research

1. Krum, S. *et al.* (2008) Estrogen protects bone by inducing Fas ligand in osteoblasts to regulate osteoclast survival. EMBO J. **27**:535.

Mouse Fas Ligand/TNFSF6 Monoclonal Antibody
(Catalog # MAB5262)
Sample: Mouse pre-osteoclasts and bone
Application: Immunohistochemistry and Neutralization

Human RUNX2/CBFA1 Monoclonal Antibody
(Catalog # MAB2006)
Sample: Mouse pre-osteoclasts and bone
Application: Immunohistochemistry

Recombinant Mouse M-CSF
(Catalog # 416-ML)
Sample: Mouse bone marrow
Application: Differentiation

2. Kominsky, S. *et al.* (2008) Macrophage Inflammatory Protein-1δ: a novel osteoclast stimulating factor secreted by renal cell carcinoma bone metastasis. Cancer Res. **68**:1261.

Human CCL15/MIP-1δ/LKN-1 Polyclonal Antibody
(Catalog # AF363)
Sample: Mouse bone marrow mononuclear cells and RAW 264.7 macrophage cell line
Application: Neutralization

Human CCL15/MIP-1δ/LKN-1 DuoSet® ELISA Development System
(Catalog # DY363)
Sample: Human bone marrow and renal cell carcinoma supernates
Application: ELISA

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Human CX3CL1/Fractalkine Polyclonal Antibody
(Catalog # AF365)
Sample: Human bone marrow tissue cores
Application: Immunohistochemistry

Human CX3CL1/Fractalkine DuoSet ELISA Development System
(Catalog # DY365)
Sample: Human bone marrow supernates and aspirates
Application: ELISA

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Human TRANCE/RANK L/TNFSF11 Monoclonal Antibody
(Catalog # MAB6261)
Sample: Human osteoblasts
Application: Flow cytometry

5. Kwak, H. *et al.* (2008) Reciprocal cross-talk between RANKL and Interferon-γ-Inducible Protein 10 is responsible for bone-erosive experimental arthritis. Arthritis Rheum. **58**:1332.

Mouse CD4 Monoclonal Antibody
(Catalog # MAB554)
Sample: Mouse bone
Application: Immunohistochemistry

Mouse CXCL10/IP-10/CRG-2 Polyclonal Antibody
(Catalog # AF-466-NA)
Sample: Mouse bone
Application: Immunohistochemistry

Mouse CXCL10/IP-10/CRG-2 Quantikine® ELISA Kit
(Catalog # MCX100)
Sample: Mouse activated $CD4^+$ T cell and $CD4^+$ T cell/osteoclast co-culture supernates
Application: ELISA

Mouse TRANCE/RANK L/TNFSF11 Quantikine ELISA Kit
(Catalog # MTR00)
Sample: Mouse activated $CD4^+$ T cell and $CD4^+$ T cell/osteoclast co-culture supernates
Application: ELISA

Mouse TNF-α/TNFSF1A Quantikine ELISA Kit
(Catalog # MTA00)
Sample: Mouse activated $CD4^+$ T cell and $CD4^+$ T cell/osteoclast co-culture supernates
Application: ELISA

Mouse IFN-γ Quantikine ELISA Kit
(Catalog # MIF00)
Sample: Mouse activated $CD4^+$ T cell and $CD4^+$ T cell/osteoclast co-culture supernates
Application: ELISA

6. Zanotti, A. *et al.* (2008) Activation of the ERK Pathway in osteoblastic cells, role of Gremlin and BMP-2. J. Cell Biochem. **104**:1421.

Recombinant Mouse Gremlin
(Catalog # 956-GR)
Sample: ST2 mouse bone marrow-derived stromal cell line and osteoblasts
Application: Blocking BMP-2-induced SMAD phosphorylation

7. Chamoux, E. *et al.* (2008) Osteoprotegerin decreases human osteoclast apoptosis by inhibiting the TRAIL pathway. J. Cell Physiol. **216**:536.

TdT *In Situ* Apoptosis TUNEL Kit - TACS® Blue Label
(Catalog # TA4626)
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Application: *In situ* detection of apoptosis

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(Catalog # 215-GM)
Sample: Human mononuclear cells
Application: Osteoclastogenesis

Recombinant Human M-CSF
(Catalog # 216-MC)
Sample: Human mononuclear cells
Application: Osteoclastogenesis

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Mouse Wnt-10b Monoclonal Antibody
(Catalog # MAB2110)
Sample: Mouse calvarial osteoblasts and long bone mesenchymal cell lysates
Application: Western blot

9. Shimizu, H. *et al.* (2008) Angiotensin II accelerates osteoporosis by activating osteoclasts. FASEB J. **22**:2465.

Human TRANCE/RANK L/TNFSF11 Polyclonal Antibody
(Catalog # AF626)
Sample: Human osteoblasts
Application: Western blot

10. Li, X. *et al.* (2008) Role of decorin in the antimyeloma effects of osteoblasts. Blood **112**:159.

Mouse Wnt-10b Monoclonal Antibody
(Catalog # MAB2110)
Sample: Mouse calvarial osteoblasts and long bone mesenchymal cell lysates
Application: Western blot

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TECHNICAL NOTE: Using R&D Systems Proteome Profiler™ Antibody Arrays

Recent Studies Investigate Pathways Mediating Drug Resistance in Cancer Cells

Since activation of a number of different signaling pathways may promote the cancer phenotype, screening tools that simultaneously assess the phosphorylation state of multiple proteins can be extremely useful. R&D Systems Proteome Profiler Human Phospho-RTK Antibody Array (Catalog # ARY001), Phospho-MAPK Array (Catalog # ARY002), and Phospho-Kinase Array (Catalog # ARY003) allow the phosphorylation of multiple RTKs, or intracellular kinases to be assessed in a single sample of cell lysate (Figure 1). Several recent high profile studies have used the Proteome Profiler Arrays to provide insight into the mechanisms by which some tumors become resistant to specific anticancer drugs.

Receptor tyrosine kinases (RTKs) are transmembrane proteins that initiate signaling pathways required for normal cellular processes such as growth and development. Mutations in RTKs can cause constitutive activation of downstream signaling pathways implicated in the pathogenesis of different forms of cancer. Therefore, monitoring RTK phosphorylation in cancer cells can be useful for the development of treatments.

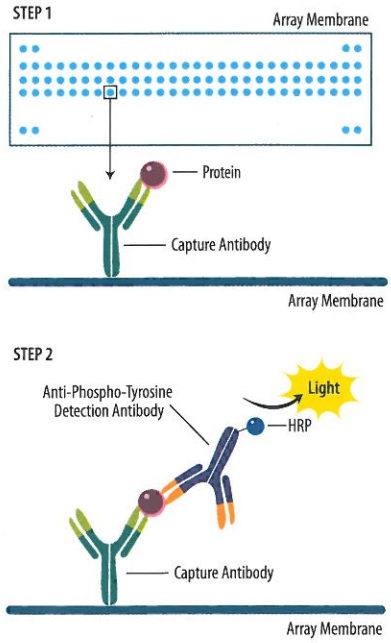

Figure 1. R&D Systems Proteome Profiler Phospho-RTK Antibody Array Detects the Phosphorylation State of Multiple Receptors in a Single Sample. Proteome Profiler Antibody Arrays are designed using carefully selected capture antibodies that are spotted in duplicate on nitrocellulose membranes. When these membranes are incubated with experimental samples, capture antibodies printed on the membranes bind to their specific target proteins (**Step 1**). Capture antibodies on the Phospho-RTK array bind the extracellular domain of both phosphorylated and unphosphorylated RTKs, and an HRP-conjugated pan anti-Phospho-Tyrosine antibody is subsequently used to specifically detect phosphorylated RTKs (**Step 2**). Proteins are visualized using chemiluminescent detection reagents which produce a signal that is proportional to the amount of analyte bound. The use of Proteome Profiler antibody arrays requires no specialized equipment and eliminates the need to perform multiple immunoprecipitation/Western blot experiments.

Scientists at the Dana Farber Cancer Institute and Harvard Medical School utilized the Phospho-RTK Antibody Array to identify phosphorylated RTKs in brain tumor glioblastoma multiforme (GBM) in an effort to determine why therapies targeting single RTKs in these tumors have failed.[1] They found that not one, but several RTKs are activated in GBM cell lines (Figure 2A) and in primary GBMs, as well as in other solid tumor cell types such as lung and pancreatic adenocarcinomas. Most phosphorylated RTKs were also activated under conditions of serum starvation and in tumor cell xenografts, indicating that RTK phosphorylation detected in cultured cells was likely not induced by factors present in the cell culture media (Figure 2B). Significantly, this study led to the finding that targeting multiple RTKs simultaneously could suppress the cancer phenotype in glioma cells far more significantly than treatments targeting single RTKs alone.

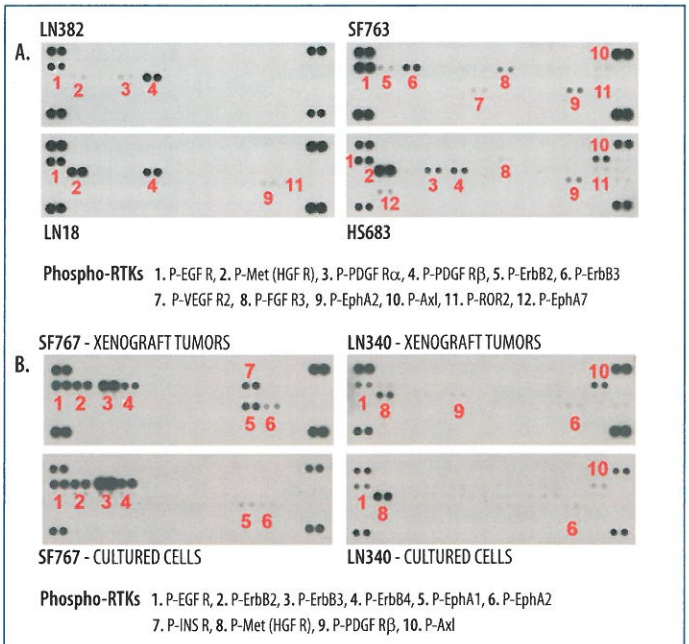

Figure 2. Detection of Activated Receptor Tyrosine Kinases (RTKs) in Glioma Cell Lines using the Proteome Profiler Human Phospho-RTK Antibody Array. **A.** Stommel *et al.* assessed the phosphorylation of 42 different receptor tyrosine kinases (RTKs) in whole cell extracts prepared from the indicated glioma cell lines using the Proteome Profiler Human Phospho-RTK Array Kit (Catalog # ARY001). **B.** RTK phosphorylation was assessed in whole cell extracts from cultured SF767 or LN340 glioma cells (bottom) or in xenograft tumors derived from these cell lines (top) using the Human Phospho-RTK Array. Activated RTKs are indicated at the bottom. [*Figure from Stommel, J.A. et al. (2007) Science* ***318****:287-290. Reprinted with permission from AAAS.*]

Other researchers at these institutions used the Phospho-RTK Antibody Array to investigate the mechanisms by which non-small cell lung cancers (NSCLCs) containing EGF R-activating mutations develop resistance to gefitinib, an EGF R kinase inhibitor.[2] Engelman *et al.* showed that ErbB3 and Met (HGF R) are phosphorylated in drug-resistant cells, while phosphorylation of both RTKs is markedly reduced in gefitinib-treated, non-resistant cells (Figure 3). Further research demonstrated that gefitinib resistance in NSCLCs is mediated by *MET* amplification and the subsequent activation of ErbB3 signaling pathways. These results suggest that Met kinase inhibitors may be useful in combination with other therapies for treating gefitinib-resistant lung cancer tumors with EGF R-activating mutations.[2]

Technical Note continued from page 6

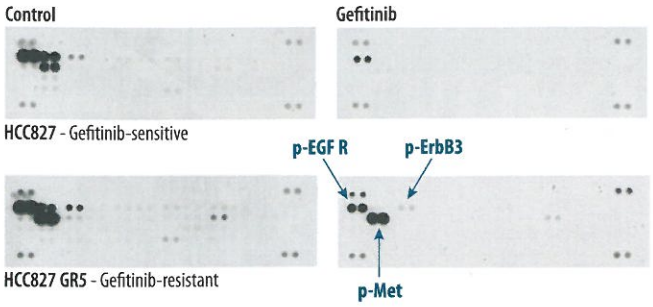

Figure 3. Detection of Phosphorylated RTKs in Gefitinib-Resistant NSCLCs. RTK phosphorylation in control (HCC827) and gefitinib-resistant (HCC827 GR5) non-small cell lung cancers (NSCLCs), in the absence (left) or presence (right) of gefitinib treatment, was determined by Engelman *et al.* using the Proteome Profiler Human Phospho-RTK Array Kit (Catalog # ARY001). Spots representing the relative levels of phosphorylated EGF R, ErbB3 and Met (HGF R) are indicated by the arrows. [*Figure from Engelman, J.A. et al. (2007) Science* ***316****:1039-1043. Reprinted with permission from AAAS.*]

A similar approach was used by Eckstein *et al.* at the Stiftung Center for Advanced European Studies and Research in Germany to identify mechanisms mediating cisplatin resistance in breast cancer.[3] Lysates from engineered cisplatin-resistant MCF-7 cells were incubated with the Phospho-RTK Array and drug resistance was found to be dependent on mechanisms involving EGF R and ErbB2 phosphorylation. Intracellular signaling molecules activated as a result of EGF R or ErbB2 phosphorylation were subsequently assessed using R&D Systems Proteome Profiler Human Phospho-MAPK Antibody Array Kit (Catalog # ARY002). This analysis revealed that ERK1 (T202/Y204) and Akt1 (S473) are highly phosphorylated in cisplatin-resistant MCF-7 cells. Further investigation demonstrated that the development of cisplatin resistance correlates with increased expression of the EGF R/ErbB ligand, Amphiregulin, which activates PI 3-K/Akt signaling pathways. These findings may open new avenues of research in the pursuit of alternative treatment options for cisplatin-resistant breast cancers.

As these recent studies show, antibody arrays simplify the screening process required to identify proteins or pathways involved in establishing specific cellular phenotypes. By monitoring changes in protein levels and/or post-translational modifications of several proteins simultaneously, molecules of interest can be rapidly identified and targeted for further study. In addition to the Phospho-RTK and Phospho-MAPK Array Kits, arrays are also available for profiling other cancer-related processes such as angiogenesis and apoptosis. Please visit our website at **www.RnDSystems.com/go/ProteomeProfiler** for more information.

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CD45 in the Stem Cell Niche continued from page 1

subsequent mobilization of normal HSCs, indicating a role for CD45 in the niche, as well as in the HSC.[4]

While G-CSF has been widely used to induce stem cell mobilization into the peripheral circulation, many other "stress signals" also have a mobilizing effect.[6] TRANCE (TNF-related activation-induced cytokine), also known as RANK L (receptor activator of NF-κB ligand) is one such mobilizing agent.[3,4] However, in CD45$^{-/-}$ mice, RANK L is no longer able to mobilize HSCs. Since osteoclasts are the main cell type in the niche that express the receptor for RANK L, this implicates osteoclast CD45 in RANK L-induced mobilization.[4] RANK L is better known as a mediator that is required for osteoclast differentiation, and this function also appears to be impaired in the CD45$^{-/-}$ mice. In addition to abnormal morphology and niche remodeling, CD45$^{-/-}$ osteoclasts show impaired G-CSF-stimulated metalloproteinase secretion, and a decrease in both RANK L-stimulated osteopontin degradation and soluble stem cell factor (SCF; c-kit ligand) release.[4] These events are known to be regulated by SFK and important for HSC mobilization.[3,7-9]

A role for CD45 in osteoclasts has not previously been shown, although its role in dephosphorylating SFK and other substrates has been demonstrated in many classes of leukocytes.[5] Several questions remain concerning CD45, including the reason for its highly regulated expression of isotypes and other differentially glycosylated forms, as well as the identity of its putative extracellular ligand.[10] However, the involvement of osteoclast CD45 in endosteal niche architecture and HSC mobilization provides new evidence that the role that osteoclasts play, whether direct or indirect, is important for HSC homeostasis.

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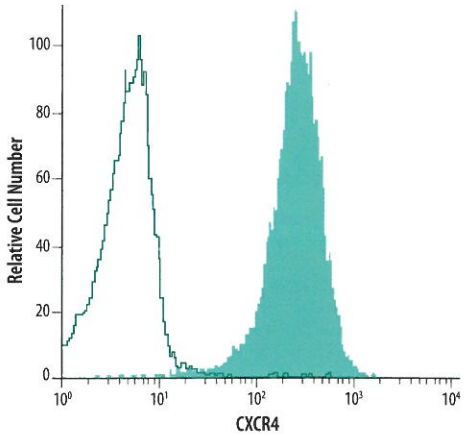

CXCR4 Expression in Jurkat Cells Using Flow Cytometry. Jurkat human leukemic T cells were labeled using PerCP-conjugated anti-human CXCR4 (Catalog # FAB170C; filled histogram) or with an isotype control antibody (Catalog # IC003C; open histogram).

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CCR7	Human	FAB197C
CD3ε	Human	FAB100C
CD4	Human	FAB3791C
CD8α	Human	FAB1509C
CD19	Human	FAB4867C
CXCR2/IL-8 RB	Human	FAB331C
CXCR3	Human, Mouse	FAB160C, FAB1685C
CXCR4	Human	FAB170C
CXCR5	Human	FAB190C
Endoglin/CD105	Human	FAB10971C
Erb3/HER3	Human	FAB3481C
IGF-I R	Human	FAB391C
IL-17/IL-17A	Human	IC3171C
LAP (TGF-β1)	Human	FAB2463C
NKG2A	Human	FAB1059C
NKG2C	Human	FAB138C
NKG2D	Human	FAB139C
Oct-3/4	Human, Mouse	IC1759C
SOX2	Human, Mouse	IC2018C
SSEA-4	Human, Mouse	FAB1435C
T-bet/TBX21	Human	IC5385C
VEGF R2/KDR/Flk-1	Human	FAB357C
Goat $F(ab)_2$ Anti-mouse IgG (H+L)	Mouse	F0114
Goat $F(ab)_2$ Anti-rat IgG (H+L)	Rat	F0115
Mouse IgG_1 PerCP Isotype Control	Mouse	IC002C
Mouse IgG_{2A} PerCP Isotype Control	Mouse	IC003C
Mouse IgG_{2B} PerCP Isotype Control	Mouse	IC0041C
Mouse IgG_3 PerCP Isotype Control	Mouse	IC007C
Rat IgG_{2A} PerCP Isotype Control	Rat	IC006C
Rat IgG_{2B} PerCP Isotype Control	Rat	IC013C