PAPP-A: A New Anti-Aging Target?

To live an extended, healthy life is a human dream that scientists are striving to make come true through a better understanding of underlying mechanisms. Cheryl A. Conover, PhD, of the Division of Endocrinology, Diabetes, Metabolism, and Nutrition at Mayo Clinic in Rochester, Minnesota, is one of those scientists. On the basis of recent studies from her laboratory, she proposes pregnancy-associated plasma protein-A (PAPP-A) as a therapeutic target for aging and age-related disease.

What Is PAPP-A?

Dr Conover explains: “PAPP-A was originally identified as 1 of 4 placently derived proteins found at high concentrations in the plasma of pregnant women, hence the name ‘pregnancy-associated plasma protein-A.’ However, the function of this protein remained elusive. Twenty-five years later, we discovered that PAPP-A was a novel zinc metalloprotease expressed by various nonreproductive cell types as well, including fibroblasts, vascular smooth muscle cells (SMCs), and osteoblasts. Our in vitro and in vivo studies indicated that PAPP-A functions to enhance the growth-stimulating effects of local insulinlike growth factors (IGFs) through cleavage of inhibitory IGF binding proteins (IGFBPs). PAPP-A is a secreted protein that tethers to the surface of cells through proteoglycan moieties [Figure 1A]. IGF bound to IGFBP-4 is unable to activate receptors. However, on cleavage of IGFBP-4 by PAPP-A, IGF is liberated from the complex in the pericellular environment and IGF signaling is initiated.”

The IGF System, PAPP-A, and Longevity

The IGFs are associated with cellular and chronological aging, and reduction of IGF-1 signaling has been shown to prolong lifespan in diverse species. Dr Conover explains: “Since PAPP-A enhances local IGF-1 action, inhibition of PAPP-A expression or proteolytic activity represents an innovative approach to decreasing IGF availability with moderate restraint of IGF-1 receptor signaling [Figure 1B]. Thus, if reduction of IGF-1 signaling prolongs the lifespan, then inhibition of PAPP-A (and consequent reduction in bioavailable IGF-1) should promote longevity. To acquire the in vivo model to test this hypothesis, I took a sabbatical with Jan van Deursen, PhD, at Mayo Clinic in Minnesota and created a mouse with the PAPP-A gene deleted—the ultimate inhibition of PAPP-A. We showed that these PAPP-A knock-out (KO) mice live 30% to 40% longer than their wild-type littermates. Histopathology indicated the maintenance of a healthy immune system, delayed occurrence of neoplasms, and reduced incidence and severity of degenerative diseases of aging in PAPP-A KO mice, which enabled them to maintain a better health status into old age than wild-type mice.”

PAPP-A and Atherosclerosis

In 2001, Dr Conover and colleagues published a paper in The New England Journal of Medicine (2001;345[14]:1022–9) with the first evidence for
PAPP-A as a marker of acute coronary syndromes, which has now been confirmed in multiple clinical trials. Dr Conover highlights: “Using immunohistochemistry, PAPP-A was present in eroded and ruptured atherosclerotic plaques from human arterial specimens. The most intense staining for PAPP-A was at the inflammatory shoulder of the ruptured plaque containing activated SMCs and macrophages. There was little or no staining for PAPP-A in stable plaques. These findings suggested that PAPP-A may be synthesized in an autocrine/paracrine fashion by activated cells in unstable plaque. We found that primary cultures of human coronary artery SMCs and endothelial cells expressed PAPP-A, which was markedly stimulated by proinflammatory cytokines. Although PAPP-A immunostaining co-localized with activated macrophages in unstable atherosclerotic plaque, we showed that macrophages do not express PAPP-A. However, PAPP-A secreted by SMCs readily binds to macrophages, as well as to SMCs. Cell-associated PAPP-A [Figure 1A], which remains proteolytically active, appears to serve to effectively promote direct interaction of the ‘freed’ IGF with receptors.”

PAPP-A is clearly associated with vulnerable plaque, but does PAPP-A have an active role in the development of atherosclerosis or in plaque progression, or both? Dr Conover’s laboratory addressed this question using the PAPP-A KO mice crossed with apolipoprotein E (ApoE) KO mice, the latter being an established murine model for studies of atherosclerosis. Dr Conover summarizes her findings: “We found that ApoE KO mice lacking PAPP-A had a 70% to 80% reduction in plaque area compared to ApoE KO mice expressing PAPP-A when fed a high-fat, Western-style diet [Figure 2]. Lesion number was not different between the 2 groups. PAPP-A deficiency appeared to delay progression of lesion development from fatty streak to more advanced plaque. Thus, loss of PAPP-A was clearly beneficial for impedance of plaque development in this mouse model of atherosclerosis.”

**Importance of Dr Conover’s Overall Program**

These and other findings in PAPP-A KO mice have led Dr Conover to propose PAPP-A as a novel drug target for promoting an extended, healthy lifespan. Dr Conover explains: “Of course, we would not be knocking out the PAPP-A gene in humans, but we are developing therapies to inhibit PAPP-A’s proteolytic activity. The long-term goal of my laboratory is to generate specific PAPP-A–targeting therapies, screen efficacy of these inhibitors in vivo, determine safety and pharmacokinetic profiles of such inhibitors, and initiate clinical trials to regulate longevity and to control complications of age-related diseases in humans.”
Skeletal Health in Adult Cancer Patients

Nearly all cancers can have clinically significant negative effects on the skeleton. Matthew T. Drake, MD, of the Division of Endocrinology, Diabetes, Metabolism, and Nutrition at Mayo Clinic in Rochester, Minnesota, says: “Cancer is a major risk factor for both generalized and local bone loss, with bone loss in cancer patients substantially greater than in the general population. Cancer-associated bone loss is due to the direct effects of cancer cells and the effects of therapies used in cancer treatment, including chemotherapeutics, corticosteroids, aromatase inhibitors, and androgen-deprivation therapy (ADT) [Figure 1]. The skeleton is also the most common site of metastatic disease, because cancer cells growing within bone induce osteoblasts and osteoclasts to produce factors that stimulate further cancer growth. Accordingly, skeletal effects have become increasingly important because of improved oncologic treatments that have enhanced both patient survival and longevity.”

Breast Cancer

Morbidity due to bone disease in patients with breast cancer can have a major impact on patient quality of life because of the often long clinical course. Bone-related complications in women with breast cancer include hypercalcemia, fractures, need for irradiation or surgery, and spinal cord compression. Placebo-controlled trials have shown that in the absence of bone-protective therapies, patients have roughly 2.2 to 4.0 skeleton-related events annually.

Dr Drake explains: “Although estrogen plays a central role in bone homeostasis, hormonal therapy–based treatment regimens to effect estrogen action at the estrogen receptor (ER) are frequently used. Tamoxifen is an ER antagonist in breast tissue that is often used for patients whose breast cancer is ER-positive and as prophylaxis in patients at high risk for breast cancer. Tamoxifen may lead to bone loss in premenopausal women. By comparison, in postmenopausal women, tamoxifen can act as an ER agonist in bone and increase bone mass. However, neither tamoxifen nor raloxifene appear to reduce fracture incidence in postmenopausal women with breast cancer. Aromatase inhibitors decrease cancer recurrence and improve disease-free survival compared to tamoxifen. These inhibitors also result in significant bone loss [Figure 1] and increased fracture incidence, as demonstrated in both the ATAC (Arimidex, Tamoxifen, Alone or in Combination) and Breast International Group 1-98 trials. Upfront, vs delayed, zoledronate therapy improved bone mineral density (BMD) in postmenopausal women receiving adjuvant aromatase inhibitor therapy in Z-FAST (Zoledronic Acid-Letrozole Adjuvant Synergy Trial). More recently, denosumab was shown to increase the time to first skeleton-related event in breast cancer patients.”

Prostate Cancer

ADT is often used for palliation in prostate cancer patients. It induces clinically significant bone loss (Figure 1), likely because of the decreased availability of testosterone for conversion to estradiol. ADT also increases fracture risk. Yet, despite these profound skeletal effects, the majority of prostate cancer patients continue to be inadequately treated for their bone loss.

Dr Drake says: “In prostate cancer, zoledronic acid is the best-studied bisphosphonate. Zoledronic acid has been shown to decrease bone loss and fracture risk, as well as to reduce bone loss induced by gonadotropin-releasing hormone agonist. More recently, denosumab was shown to increase BMD in prostate cancer patients receiving ADT and to prolong the time to first skeleton-related event relative to zoledronic acid.”

Monoclonal Gammapathies

Myeloma is the second most common hematologic cancer, accounting for 10% of all hematologic cancers. Patients have both generalized bone loss and focal osteolytic lesions. Nearly
two-thirds of patients with myeloma have bone pain at presentation, and fracture rates are increased 16-fold relative to the general population in the year preceding diagnosis. Even with disease remission, skeletal lesions rarely heal.

Dr Drake highlights: “Both pamidronate and zoledronate are approved by the US Food and Drug Administration for the treatment of myeloma-related bone disease and have been shown in placebo-controlled trials to reduce hypercalcemia, bone pain, and fracture incidence. Importantly, patients with monoclonal gammopathy of undetermined significance (MGUS), a common premalignant condition with an approximately 1% annual risk of progression to myeloma, have substantially increased fracture rates. We recently demonstrated that, relative to matched control subjects, patients with MGUS have decreases in both cortical and trabecular thickness, as well as increases in circulating cytokines known to increase osteoclast activity and suppress osteoblast activity. Together, these findings may provide insight into the increased fracture incidence seen in MGUS.”

Potential Complications of Pharmacologic Therapies
Osteonecrosis of the jaw (ONJ) is now a well-recognized potential complication of antiresorptive therapy. Dr Drake notes: “Risk factors for ONJ include invasive dental procedures, poor oral hygiene, and prolonged antiresorptive therapy. Initial results suggest that rates of ONJ are comparable between zoledronate and denosumab in cancer patients. Preventative dental measures in high-risk patients [Figure 2] can substantially reduce risks; conservative management with oral rinses, pain control, antibiotics, and limited surgical intervention will lead to healing in most cases. Although now recognized as very uncommon events in osteoporosis patients, atypical femoral fractures are currently limited to rare case reports in the oncologic literature. Hypocalcemia is most common in patients receiving intravenous bisphosphonate therapy; rates appear similar between zoledronate and denosumab. Risk can be limited by optimizing the intake and levels of calcium, vitamin D, and magnesium.”

Other Factors for Optimizing Skeletal Health
In addition to pharmacologic interventions, conservative measures are extremely important for cancer patients in limiting fracture risk. Dr Drake advises: “Conservative measures include counseling about high-risk activities (falls and heavy lifting), the introduction of appropriately supervised physical therapy for muscle strengthening to limit fall risks, and ensuring adequate calcium and vitamin D intake after resolution of any existing hypercalcemia. Finally, it is important to recognize that cancer patients are often at increased fall risk due to the use of analgesics and sedatives. In conclusion, careful monitoring of bone health must be an essential component of any cancer treatment plan.”

Figure 2. Effect of preventive dental measures. These measures can substantially reduce (in this case, reduced by 78%) the incidence of osteonecrosis of the jaw in cancer patients receiving high-dose intravenous bisphosphonate therapy. Adapted from Ripamonti CI, Maniezzo M, Campa T, et al. Decreased occurrence of osteonecrosis of the jaw after implementation of dental preventive measures in solid tumor patients with bone metastases treated with bisphosphonates. The experience of the National Cancer Institute of Milan. Ann Oncol. 2009;20(1)137-45. Used with permission.
Diabetes mellitus (DM) is responsible for substantial morbidity and death, with costs of about $170 billion per year in the United States. Therefore, preventing DM is an important goal. Adrian Vella, MD, of the Division of Endocrinology, Diabetes, Metabolism, and Nutrition at Mayo Clinic in Rochester, Minnesota, says: “The states of impaired fasting glucose (IFG) and impaired glucose tolerance (IGT) are associated with a high rate of progression to type 2 DM. However, the risk is heterogeneous. For example, in Olmsted County, Minnesota, 40% of people with a fasting plasma glucose level of 110 mg/dL or greater progress to overt diabetes within 10 years, as opposed to 5% of those with a fasting plasma glucose level of less than 95 mg/dL.”

Questions for Dr Vella and his team include the following: Which patients with IFG will have DM eventually? Why does diabetes not develop in about 60% of people with IFG within 10 years, and why is there heterogeneity of pancreatic β-cell function in patients with prediabetes? Dr Vella answers: “It is clear that diabetes arises out of a complex interaction between genes and the environment. A variant in TCF7L2 confers the strongest genetic predisposition to DM and is associated with progression to DM from the prediabetic state. However, the mechanisms by which this occurs are still unidentified. TCF7L2 encodes a transcription factor that regulates proglucagon expression in the gut. Hence, it was assumed that this gene predisposed to diabetes through changes in incretin hormone secretion and, perhaps, β-cell responsiveness to incretins. However, in a study examining glucagon-like peptide-1, or GLP-1, secreted in response to an oral challenge, we demonstrated that the diabetes-associated allele of TCF7L2 did not alter concentrations of active and total GLP-1. Moreover, in a separate cohort, insulin secretion in response to pharmacological concentrations of GLP-1 was likewise unaffected by TCF7L2. Other diabetes-associated variants in WFS1 and KCNQ1 also do not alter these parameters.

Intriguingly, variation in GLP1R alters response to infused GLP-1, but the clinical significance of this in predicting response to incretin-based therapy is unknown. More importantly, GLP-1 secretion does not seem to decrease as β-cell function decreases in prediabetes—implying that defects in GLP-1 secretion do not play a part in the pathogenesis of DM.”

Dr Vella continues: “In an Olmsted County cohort of persons without DM, we have observed a parallel decrease in insulin secretion and action as fasting and postprandial glucose concentrations rise. At present, we are actively exploring mechanisms, such as the timing and amplitude of insulin secretion, that might explain this coupling. Persons with diabetes-associated common genetic variation seem to be particularly affected by this coupling of insulin secretion and action.”

Another active area of research for Dr Vella’s laboratory is the remission of type 2 DM associated with bariatric surgery for medically complicated obesity. Dr Vella explains: “The surgery that is most commonly undertaken for this purpose is Roux-en-Y gastric bypass, with remission rates of about 80% for type 2 DM. In such circumstances, caloric restriction, incretin hypersecretion in response to increased delivery of calories to the hindgut, vagal denervation, and, perhaps, altered bile acid kinetics and secretion may all play a role in the remission of DM. Moreover, remission—or lack thereof—after such surgery again provides an opportunity to examine the heterogeneity of β-cell function present in such patients and may provide further insights into the plasticity, or otherwise, of islet function at various stages of metabolic disease.”
Graves’ ophthalmopathy (GO) is an inflammatory autoimmune disorder of the orbit that primarily affects patients with a history of Graves’ hyperthyroidism. However, it is also seen in euthyroid and hypothyroid individuals who have never been hyperthyroid. Rebecca S. Bahn, MD, of the Division of Endocrinology, Diabetes, Metabolism, and Nutrition at Mayo Clinic in Rochester, Minnesota, explains: “It’s clear that autoantibodies directed against the thyrotropin (TSH) receptor, termed TRAb, cause Graves’ hyperthyroidism by stimulating the thyroid to produce excess thyroid hormones. TRAb can be detected using sensitive assay systems in essentially all patients with GO, including those without a history of hyperthyroidism. Levels of TRAb correlate with the severity and inflammatory activity of GO, and the high titers of these antibodies predict a worse prognosis. Although the onset of GO occasionally precedes or follows the onset of hyperthyroidism by many years, these conditions most commonly are diagnosed simultaneously or within about 18 months of each other. Because of the close clinical relation between Graves’ hyperthyroidism and GO, investigators have long hypothesized that both autoimmune conditions derive from a single systemic process and share the TSH receptor as a common autoantigen.”

Dr Bahn continues: “Our laboratory is interested in unraveling the pathogenesis of GO because a better understanding of the disease will enable development of novel and improved forms of therapy for this debilitating condition. The GO-affected orbit is characterized by edema, hyaluronic acid (HA) accumulation, and increased volume of adipose tissue and extraocular muscles. The muscle enlargement is in part due to the development of new fat cells within the orbital tissues. The muscle enlargement is produced as hydrophilic HA and edema collects within the connective tissues lying between the intact muscle fibers. The increase in tissue volume within the bony orbit displaces the globe forward and hinders venous outflow. As a result, cytokines and other mediators of inflammation, produced by infiltrating mononuclear cells and resident macrophages, accumulate within the orbit and contribute to the local inflammatory process.”

Dr Bahn further reports: “We have shown that TSH receptor is highly expressed in GO orbital tissues and is found specifically on the resident fibroblasts. Higher levels of TSH receptor expression can be measured in orbital fibroblasts from GO patients than in fibroblasts from normal orbits or other parts of the body. Although some orbital fibroblasts are adipocyte precursor cells that further increase TSH receptor expression as they differentiate into mature adipocytes, others are capable of producing HA in large quantities. These and other findings suggest that orbital fibroblasts are the target cells in GO and that stimulation of TSH receptor on these cells by circulating TRAbs may contribute to the tissue remodeling characteristic of the disease.”

An important question is whether TSH receptor activation by TRAbs might impact new fat cell development. To address this question, Dr Bahn says: “We treated GO fibroblasts with a high-affinity human stimulatory monoclonal antibody directed against TSH receptor (termed M22). We found that M22 stimulates not only cyclic adenosine monophosphate (cAMP) production in orbital fibroblasts (as it does in thyrocytes), but also activates phosphoinositide-3-kinase (P13K) pAkt/mTOR signaling. In doing so, it acts as a pro-adipogenic factor to increase expression of adipogenes.

**Figure.** Role of the thyrotropin (TSH) receptor in the immunopathogenesis of Graves’ ophthalmopathy. Circulating TSH receptor stimulatory autoantibodies recognize the receptor on fibroblasts residing within the orbit. Ligation of the receptor results in activation of the phosphoinositide-3-kinase (P13K)/Akt signaling cascade, as well as others, including the adenylyl cyclase (AC)/cAMP pathway. This leads to increased production of hyaluronic acid (HA) by these cells and the development of new fat cells from a subset of adipocyte precursors. In addition, insulin-like growth factor (IGF-1) and other growth factors and cytokines within the orbit may modulate these processes. This results in accumulation of HA and edema within the orbit and expansion of the orbital fat volume, leading to the varied clinical expressions of the disease. From Iyer S, Bahn R. Immunopathogenesis of Graves’ ophthalmopathy: the role of the TSH receptor. Best Pract Res Clin Endocrinol Metab. 2012 Jun;26(3):281-9. Used with permission.
genes found in late stages of adipogenesis and promotes lipid accumulation within the cells. This action was reversed when inhibitors of this pathway were introduced into the laboratory cultures. Similarly, insulinlike growth factor 1 (IGF-1), which is present in high levels within the GO orbit, acts to stimulate adipose cell development in orbital preadipocyte fibroblasts. In other studies, we showed that M22, other stimulatory TRAbs, and IGF-1 also increase HA synthesis in GO orbital fibroblasts. These findings help to explain the orbital tissue changes characteristic of GO [Figure]. In addition, they suggest that inhibition of TSH receptor signaling might block disease-related effects of TRAbs on orbital fibroblasts and may thus represent a novel approach to therapy.

Drs Susanne Neumann and Marvin C. Gershengorn of the Clinical Endocrinology Branch, National Institutes of Health, in Bethesda, Maryland, have recently developed low-molecular-weight antagonists of thyrotropin-and TRAb-stimulated TSH receptor signaling. Dr Bahn explains: “These compounds, modeled after similar antagonists of luteinizing hormone and follicle-stimulating hormone, were developed using high-throughput screening and functional experiments. Acting as allosteric modulators, they sit within the transmembrane portion of TSH receptor and prevent activation of the receptor without interfering with TSH or TRAb binding to the receptor. In studies using human thyrocytes, these compounds have been shown to inhibit cAMP production stimulated by immunoglobulins from the sera of patients with Graves’ hyperthyroidism. Because these small drug-like compounds are not degraded in the gastrointestinal tract, they carry potential as oral agents for the treatment of both Graves’ hyperthyroidism and GO. We are currently collaborating with Drs Neumann and Gershengorn to study these TSH receptor antagonists in our tissue culture model of GO. Our early studies have revealed a 50% to 70% decrease in cAMP production, Akt phosphorylation, and HA synthesis in cells treated with both a stimulatory TRAb and a TSH receptor antagonist, compared with cultures containing the TRAb alone.”

Dr Bahn summarizes: “GO is a debilitating ocular disease for which no uniformly effective therapy exists at present. Recent insights into the important role of TSH receptor and TRAb in the development of the tissue remodeling characteristic of the disease suggest new approaches to therapy. Rather than focusing primarily on suppression of the autoimmune process itself, we are directing efforts toward understanding the impact of small drug-like TSH receptor antagonists on orbital fibroblast functions relevant to disease development. These compounds hold promise not only because they are novel for established GO, but also because they could pertain to both the treatment of hyperthyroidism and the prevention of ocular changes in patients with Graves’ disease.”
Education Opportunities

13th Annual Mayo Clinic Nutrition and Wellness in Health and Disease
Nutrition, physical activity, and other healthy lifestyle behaviors are vital components in the promotion of health and the treatment of disease. This course—designed for physicians, advanced practice clinicians, dietitians, nurses, and health and wellness staff—will provide a full-spectrum, in-depth overview of situations that clinicians encounter in the ambulatory setting. The topics include obesity, obesity-associated medical conditions, effective ways to provide coaching, and nutrition for selected groups, in addition to physical activity and wellness. Current clinical topics will be highlighted through presentations, interactive case studies, and panel discussions. The course will be held at the Westin Michigan Avenue in Chicago. For more information about this course, please call 800-323-2688 or visit www.mayo.edu/cme/endocrinology.

17th Mayo Clinic Endocrine Course
Designed for endocrinologists and interested internists and surgeons, the 17th Mayo Clinic Endocrine Course will address gaps in medical knowledge and barriers in clinical practice, in order to improve the outcomes of patients with endocrine and metabolic disorders. This course will span the full range of endocrinology through lectures, debates, panel discussions, clinicopathologic sessions, “clinical pearls” sessions, informal breakfast round table discussions, and small-group discussions with experts. Attendees will have plenty of opportunity for interaction with the course faculty, who are selected from Mayo Clinic for their expertise and clinical acumen. An optional thyroid ultrasonography course will also be offered. For more information about this course, including location and dates, please call 800-323-2688 or visit www.mayo.edu/cme/endocrinology.

New Staff in Endocrinology at Mayo Clinic in Minnesota
Two endocrinologists joined the Division of Endocrinology, Diabetes, Metabolism, and Nutrition at Mayo Clinic in Rochester, Minnesota, during 2012. They (and their areas of interest) are Alice Y. Chang, MD, (polycystic ovarian syndrome) and Vinaya Simha, MBBS, MD, (lipodystrophy and hyperlipidemia).

Nutrition Support Service at Mayo Clinic in Minnesota
The Adult Nutrition Support Service at Mayo Clinic in Rochester, Minnesota, (Saint Marys Hospital and Rochester Methodist Hospital) will receive the inaugural Clinical Nutrition Team of Distinction Award from the American Society of Parenteral and Enteral Nutrition (ASPEN). The team of endocrinologists, dietitians, nurses, and pharmacists provides consultative care for adult hospitalized patients receiving parenteral nutrition and enteral tube feeding. The award recognizes “excellence in interdisciplinary clinical nutrition practice in institutions who meet certain criteria and compliance with ASPEN national standards, guidelines, and values.”