

## Nothing but skin and bone

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### Review Series

Skin and bone — what comes to mind at hearing this phrase? While certainly a metaphor for disease, it also defines two very different tissues, one a flexible and contiguous outer covering, the other a morphologically diverse hard tissue distributed at over 200 sites in the body. As the accompanying series of Reviews highlights, these tissues are indeed diverse, but there are also surprising similarities. Skin is the interface between the internal organs and the environment, and as such plays a crucial role in the body's defense mechanism. The skin and its many appendages are responsible for functions as diverse as epidermal barrier and defense, immune surveillance, UV protection, thermoregulation, sweating, lubrication, pigmentation, the sensations of pain and touch, and, importantly, the protection of various stem cell niches in the skin. Bone serves a number of purposes: it provides protection for vital organs, a lever for locomotion, a reservoir for calcium, and the site of adult hematopoiesis. The tissue is composed of osteoblasts, osteoclasts, and their individual precursors plus a complex mixture of mesenchymal, myeloid, and lymphoid cells in the marrow space. Finally, the endothelial microenvironment provides nutrition and is a conduit for the influx and emigration of cells that impact bone biology in several important ways. This Review series guides the reader through these various facets of 2 diverse, [...]

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**Skin and bone — what comes to mind at hearing this phrase? While certainly a metaphor for disease, it also defines two very different tissues, one a flexible and contiguous outer covering, the other a morphologically diverse hard tissue distributed at over 200 sites in the body. As the accompanying series of Reviews highlights, these tissues are indeed diverse, but there are also surprising similarities. Skin is the interface between the internal organs and the environment, and as such plays a crucial role in the body's defense mechanism. The skin and its many appendages are responsible for functions as diverse as epidermal barrier and defense, immune surveillance, UV protection, thermoregulation, sweating, lubrication, pigmentation, the sensations of pain and touch, and, importantly, the protection of various stem cell niches in the skin. Bone serves a number of purposes: it provides protection for vital organs, a lever for locomotion, a reservoir for calcium, and the site of adult hematopoiesis. The tissue is composed of osteoblasts, osteoclasts, and their individual precursors plus a complex mixture of mesenchymal, myeloid, and lymphoid cells in the marrow space. Finally, the endothelial microenvironment provides nutrition and is a conduit for the influx and emigration of cells that impact bone biology in several important ways. This Review series guides the reader through these various facets of 2 diverse, yet interdependent, tissues.**

While in the adult vertebrate organism, bone and skin spend much of their time as separate entities with vastly different agendas, the skin dermis and the bone originate from a common primordial mesenchyme, and at some points in development the overlying epidermis — in the form of the apical ectodermal ridge — and the outgrowth of the limb are intimately interdependent. In the earliest days of development, and again in times of need such as limb regeneration in tetrapod vertebrates, the 2 tissues must come together and function very much as one. The study of developmental pathways and epithelial-mesenchymal interactions in the skin and bone have revealed some striking parallels, which are reprised in both organs in the adult.

### The skin you're in: don't be fooled by a pretty exterior

Despite its aesthetically pleasing appendages such as the hair and nails, its pliable nature, its flexibility, and its responsiveness, the skin is a master in the art of self-defense — proving that a tissue need not be hard in order to be tough. In addition to serving as the body's outermost protective covering, the skin barrier integrates the body's physiology with the terrestrial environment. The epidermal barrier works in 2 ways: as an inside-out barrier, to minimize transepidermal water loss, and as an outside-in barrier, as a sentry to prevent invasion by infectious agents and noxious substances (1–5).

In addition to the 2 major structural layers of the skin, the epidermis and the dermis, the skin is also home to a number of other cell types and structures that each play a unique role in its function (6). There are resident dendritic cells, known as Langerhans cells, which are the antigen-presenting cells of the skin interspersed among the keratinocytes that provide the first

line of defense against invasion. Mast cells reside in the dermis, and their degranulation releases vasoactive amines and other proinflammatory mediators that induce immediate hypersensitivity responses such as urticaria (7).

The skin is also populated by eccrine (sweat) glands, which allow for temperature regulation via sympathetic nervous system control of an intricate network of lymphatic and blood capillaries that reside in the dermis, as well as the apocrine (scent) glands, which are believed to emit secretions and pheromones for sexual communication (8). The sebaceous gland is attached to the hair follicle and secretes sebum to the skin surface that keeps it supple and waterproof (9). The hair follicle, in addition to generating the hair shaft, also provides a protective niche to several stem cell populations in the skin, including the keratinocyte stem cell, the melanocyte stem cell, a population of epidermal neural crest stem cells, and the dermal stem cell compartment, known as the dermal papilla (10–13). There are several contractile cell populations, such as the myoepithelial cells lining the sweat gland, that contract to extrude liquid onto the skin surface during thermoregulation, as well as the arrector pili muscle, which attaches to the hair follicle and is responsible for creating goose bumps when the body's core temperature falls (14).

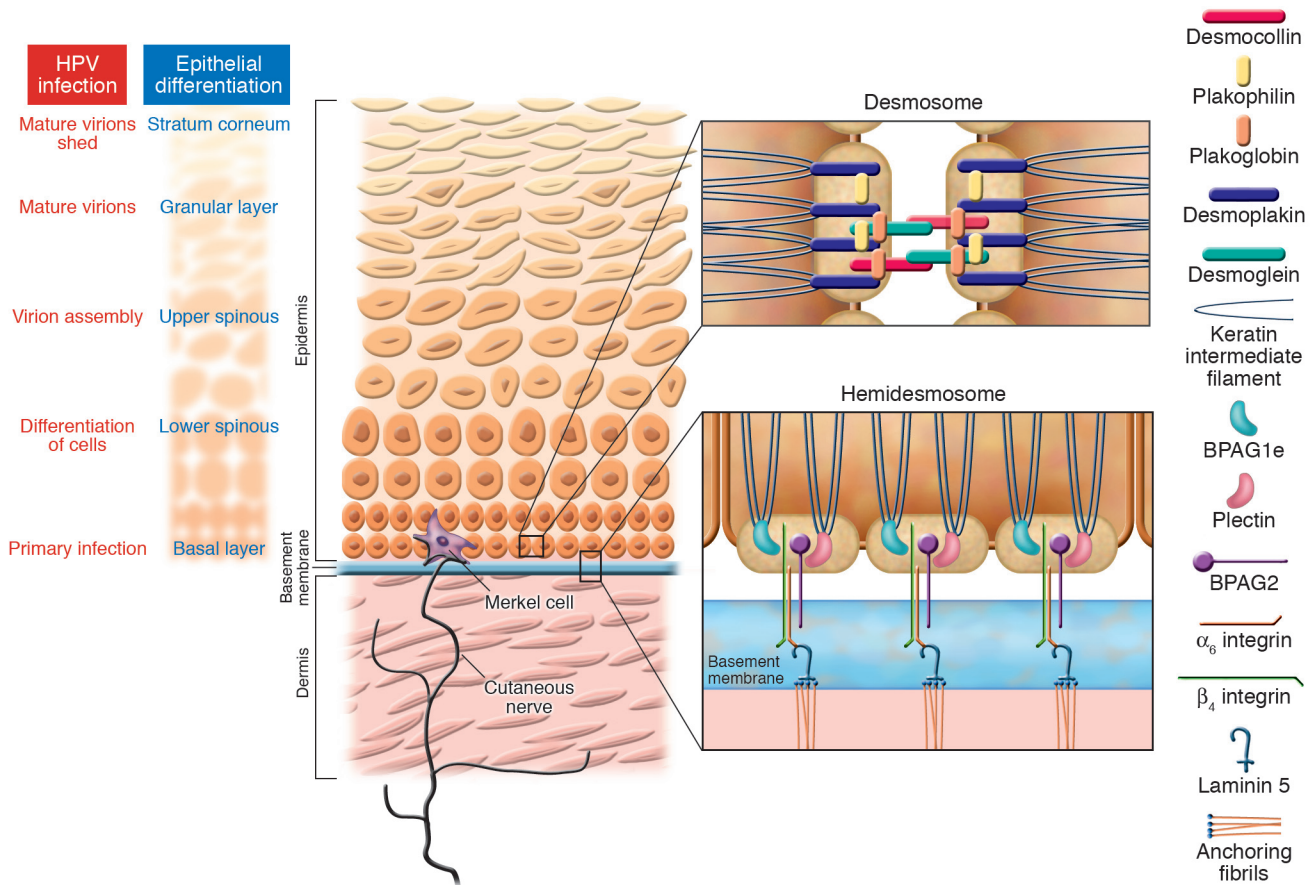
Adult skin is also home to at least 2 different neural crest cell populations: the melanocyte, which provides pigmentation and UV protection to human epidermis and color to the hair shaft (15), and the Merkel cell, a neuroendocrine cell responsible for transmission of touch sensation through the cutaneous nerves, among other functions (Figure 1) (16). There are also significant anatomical variations in skin structure on different parts of the body, such as the thick, protective epidermis on the palms and soles compared with the thin skin on the eyelid, and specialized regions without hair follicles, such as the glabrous (lip) epithelium, or with a high density of hair follicles, such as the scalp.

Thus the skin you're in is a highly specialized and meticulously regulated organ system populated by numerous different cell types that each contribute uniquely to its multitude of functions (6). Likewise, the spectrum of skin disorders that arise when these functions go awry is virtually limitless. The skin-related Reviews

**Nonstandard abbreviations used:** BMP, bone morphogenic protein; BP, bullous pemphigoid; Dkk, Dickkopf; HPV, human papillomavirus; LRP, LDL receptor-related protein; MIP-1 $\alpha$ , macrophage inhibitory protein 1 $\alpha$ ; OPG, osteoprotegerin; PTH, parathyroid hormone; PTHrP, PTH-related protein; PV, pemphigus vulgaris; RANKL, receptor activator of NF- $\kappa$ B ligand; TCF/LEF, T cell factor/lymphoid enhancer binding factor.

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**Figure 1**

Schematic illustration of several key elements of the skin. As indicated, the skin is divided into 2 main compartments, epidermal (tan) and dermal (pink), separated by a basement membrane (blue). The epidermis serves as the protective barrier, due to the differentiation of proliferative epithelial cells in the basal layer to the terminally differentiated cells in the stratum corneum. The sites and development of HPV infection are indicated at far left: the primary infection occurs in the basal layer, and mature virion sheds in the stratum corneum. Merkel cells are located within the basal layer of the epidermis (purple) and are associated with sensory nerve endings (brown). Two types of cell junctions critical for the integrity of the skin are highlighted here. Desmosomes (upper right inset) form cell-cell junctions, in which cadherins (pink and green), such as desmogleins 1 and 3, are the extracellular bridges and the autoantigens in different forms of pemphigus. Hemidesmosomes (lower right inset) tether the cells to the basement membrane and are composed of a number of proteins, 2 of which — BP230 and BP180 (also known as type XVII collagen) — are autoantigens in BP. Disruption of these interactions results in loss of adhesion of the cells to one another or to the underlying basement membrane.

in this series focus on 4 main topics: the epidermal barrier, autoimmune skin disease, epithelial viral infection, and the relationship between the skin and the central nervous system.

### When the flesh is weak

The series begins with a Review of epidermal barrier formation by Julia Segre (17). In addition to serving as the body's outermost protective covering, the skin is a barrier between the body and the terrestrial environment. Barrier function is critical in newborn animals, as shown by transgenic animal models with barrier defects that die shortly after birth from transepidermal water loss.

Early studies of the skin barrier focused mainly on its remarkable physiochemical properties and on determining the unique protein composition of lipids and the cornified cell envelope

(1, 2, 4, 5). Genetic approaches and efforts in genomic cloning have revealed an unusually complex cluster of genes in human chromosome 1q21, termed the epidermal differentiation complex, which contains more than 30 genes involved in terminal differentiation of the skin (18).

Recent studies of the molecular mechanisms governing barrier formation, particularly transcriptional events, have begun to shed light on the molecular underpinnings of this highly regulated process. In her Review (17), Segre draws some intriguing parallels between the formation of the epidermal barrier and the reprisal of these processes in the setting of common skin disorders such as psoriasis or atopic dermatitis, which display a perturbation in barrier function that may exacerbate these conditions. Taking clues from the transcriptional and molecular events involved in



## review series introduction

forming the barrier in utero, this Review proposes that the management of inflammatory skin disease may benefit from therapies that enhance restoration of the epidermal barrier.

### A “multiple hit” model for the pathogenesis of pemphigus: Tregs to the rescue

The next article in the series is a Review by Michael Hertl and colleagues (19), who offer a new perspective on the pathogenesis of a family of rare, autoimmune blistering skin diseases known as pemphigus and pemphigoid. The name pemphigus is derived from the Greek word *pemphix*, meaning “bubble” or “blister,” originally coined by McBride in 1777 and Wichmann in 1791. The more severe forms of pemphigus are potentially life threatening and involve the skin as well as the oral mucosa (20).

The autoantigens in pemphigus are epidermal desmosomal cadherins that mediate the intercellular adhesion of keratinocytes, specifically desmoglein 1 in pemphigus foliaceus and desmoglein 3 in pemphigus vulgaris (PV). Likewise, the autoantibodies in bullous pemphigoids (BPs) are 2 hemidesmosomal proteins that are involved in cell basement membrane attachment of basal keratinocytes, specifically the 180-kDa BP antigen (BP180, also known as type XVII collagen) and the 230-kDa BP antigen (BP230).

The pathognomonic blisters of pemphigus arise from the binding of circulating autoantigenic IgG molecules to these keratinocyte proteins and the subsequent disruption of intercellular adhesion either through a direct effect on desmosomal or hemidesmosomal architecture, by triggering cell signaling processes that result in loss of cell adhesion (acantholysis), or both. Thus the critical role of the B cell in generating the secreted autoantibodies found in pemphigus patients has long been known. Hertl and colleagues focus instead on the emerging importance of the T cell in pemphigus (19). These authors describe a “multiple hit” model of loss of immune tolerance in pemphigus. The authors review several lines of evidence that collectively show that neither circulating IgG nor autoreactive T cells alone are sufficient to elicit PV and suggest that a third population of cells, Tregs, may be critical in its pathogenesis.

The role of Tregs in suppressing immune responses, controlling autoimmunity, and maintaining tolerance has not been widely studied in the setting of pemphigus; however, some recent work suggests a potential imbalance of these cells in PV patients. One study found that Tregs were found in the autoreactive T cells of a large number of healthy individuals but in only 20% of PV patients, raising the possibility that the Tregs were required to keep the autoreactive T cells from evoking disease. A multiple hit model in pemphigus would therefore require an autoreactive B cell, an autoreactive T cell, and finally a loss of Treg suppressor activity in order to set the stage for PV. The requirement of this rare constellation of events might explain the relatively low population incidence of PV, at only 1–2 cases per 100,000 individuals. Hertl and colleagues suggest that focusing on Tregs may represent a new therapeutic opportunity in the restoration of immune tolerance in the pemphigus family of autoimmune bullous diseases.

### The end of a scourge: HPV no more

The next Review, by Douglas Lowy and John Schiller (21), summarizes recent developments in understanding human papillomavirus (HPV) and the development of successful HPV vac-

cines. HPVs are a large family of double-stranded DNA viruses that are virtually ubiquitous and infect epithelial tissues including the skin, cervix, and other mucosae. Over 75 variants have been identified from human tissues, about one-third of which are sexually transmitted and give rise to a spectrum of lesions within the genital tract. Some HPVs, such as HPV6 or HPV11, cause only benign epithelial lesions such as genital warts, while others, such as HPV16 and HPV18, result in lesions that can progress to invasive cancers of the cervix (22). HPVs that infect the cutaneous epithelium have also recently been linked as a cofactor along with UV exposure in the development of non-melanoma cancer of the skin (23).

Primary infection of HPV in skin or cervical epithelia usually occurs within the long-lived basal stem cells, wherein the virus replicates and immediate early proteins are expressed. As the epithelial cells begin to differentiate, the viral proteins E6 and E7 are expressed. The L1 and L2 proteins are not expressed until the virus reaches the upper spinous layers, where assembly occurs, and finally the production of mature virions takes place in the stratum corneum, from whence intact virions are shed (24).

Initial HPV exposure occurs during sexual activity, where the infection of the cervical epithelium leads to a squamous intraepithelial lesion caused by either a low-risk HPV (e.g., HPV6 and HPV11) or a high-risk HPV (e.g., HPV16, -18, -31, -33, -45). Increased sexual activity and the total number of partners results in a higher lifetime risk of HPV exposure. Similarly, in oral mucosa, high-grade HPV lesions can be promoted by smoking and other factors and result in invasive squamous cell carcinomas over long periods of time. Although such occurrences are rare, low-grade squamous intraepithelial lesions may evolve into invasive squamous carcinomas over time.

Cervical cancer comprises 10% of all cancers in women and is the second leading cause of death from cancer among women worldwide. HPV DNA is found in over 90% of cervical cancer lesions. Roughly 80% of all cervical cancers occur in less-developed countries, largely due to insufficient resources for cervical cancer screening (e.g., Pap smear). This striking association suggests the possibility of developing either prophylaxis or new therapies for cervical cancer based on the manipulation of human immune responses against HPVs.

Recently, Merck and GlaxoSmithKline announced stunning successes in the development of vaccines against HPVs. These vaccines were developed against the L1 capsid protein, which induces high levels of antibodies. Both vaccines target HPV16 and HPV18, which make up more than 70% of cancers, and the Merck vaccine also targets HPV6 and HPV11, which account for about 90% of genital warts. These vaccines have shown nearly 100% efficacy in the prevention of HPV infection in women. In their Review, Lowy and Schiller (21) address many important considerations in the design and testing of these vaccines, including public health and ethical issues. These vaccines have great potential to prevent several hundreds of thousands of cancers each year, most of which occur in young, sexually active women.

The development of HPV vaccines represents a powerful illustration of translational research whereby advances in epithelial virology can move into the commercial sector for the development of eventual large-scale vaccination programs. Perhaps successes such as the one described by Lowy and Schiller for HPV will carry over into similar programs for herpesvirus infections, a most important cause of morbidity in human populations.



### Scratch that itch

To broaden our thinking about the intimate connection between the skin and the nervous system, the final skin-related Review in this series, by Ralf Paus and colleagues (25), raises some intriguing new possibilities in managing a symptom unique to dermatology: pruritus, or itching. Despite many years of research, the exact cause of itching is unknown and represents a complex physiological phenomenon. An itch can be defined as an unpleasant sensation that provokes an often uncontrollable desire to scratch, which ironically can be inexplicably pleasurable. Scratching is believed to relieve the itch by inducing mild pain that causes a temporary distraction from the itch. Frequently, one worsens the other, since damage to the outer layers of the skin by scratching can release additional proinflammatory agents that further exacerbate itching. This phenomenon is known as the itch-scratch cycle, which lies at the center of current neurophysiological research in the skin (26).

Pruritus can be a symptom of innumerable skin diseases; however, it can also occur when there is no visible evidence of a skin lesion and instead results from a disruption in processing of the itch sensation in the brain or the circuitry connecting it with the skin. There are many systemic diseases that can cause itch, including kidney failure, hepatitis C infection, multiple myeloma, and liver disease, among others, suggesting that stimuli from outside the skin-brain circuit can enter the pathway and cause itching.

Pruritus has been a challenge from the research perspective due to the subjective nature of itching itself, the absence of a precise physiological definition or quantitative measures, and the lack of suitable *in vitro* or *in vivo* models to mimic the symptoms. Despite these challenges, recent advances in understanding the neurophysiological basis of itch have revealed some surprising new insights. The skin is home to a complex network of nerves that transmit different sensations, including itch, pain, touch, cold, and heat. In the simplest of terms, itching is a response to a chemical stimulus that transmits these signals back to the brain (27). Teasing out which nerve fibers are responsible for itch has been a formidable task, and for many years it was believed that itch simply hijacked nerve circuits from the pain pathways and was merely a modified form of pain. However, the discovery of itch-specific neurons revealed that there are, in fact, distinct sensory systems for itch and pain (nociception). In their Review (25), Paus and colleagues discuss some potentially novel therapeutic opportunities that have arisen from a better understanding of itch from the point of view of the skin as well as the brain.

### What lies beneath

The skin-related Reviews within this series focus mainly on 4 variations on the theme of the epithelial cutaneous disease, since the epidermal barrier, the destruction of keratinocytes in pemphigus, viral infection of epithelial cells by HPV, and the origins of itching all relate in some way to the epidermis or its resident cells. There is far more to the skin than the epidermis, and to consider it in isolation would be just scratching the surface. The dermis, home of mesenchymal cells in the skin, was not touched upon extensively in these Reviews, nor was the vast subject of developmental pathways such as Wnt signaling (reviewed in refs. 28, 29). Interestingly, in early development, undifferentiated mesenchymal cells can give rise to the fibroblasts of skin dermis, to adipocytes, to cartilage, to muscle, or to bone, suggesting that there are common themes at play in

differentiation of these tissues (30). Since mesenchymal cells and signaling pathways are the primary focus of the second half of this Review series, we shall now cut straight to the bone and examine what lies beneath.

### Bone

Physiological bone turnover can be divided into 2 temporal phases: modeling, which occurs during development (a topic not addressed in this series; for recent reviews see refs. 31, 32), and remodeling, a lifelong process involving tissue renewal. Remodeling starts with removal by osteoclasts of matrix, a mixture of insoluble proteins in which type I collagen is predominant (>90%) and a poorly crystalline, chemically modified hydroxyapatite. Following resorption, osteoblasts are recruited to the site, where they secrete and mineralize new matrix. Until about age 30–35 bone replacement exceeds or equals removal, thus increasing or maintaining bone mass; thereafter, bone mass decreases, reflecting the predominance of osteoclast activity. The major thrust of the bone-related Review articles contained within this series is to outline selected new and important aspects of osteoblast and osteoclast biology (for an excellent review of the pathophysiology of osteoporosis see ref. 33).

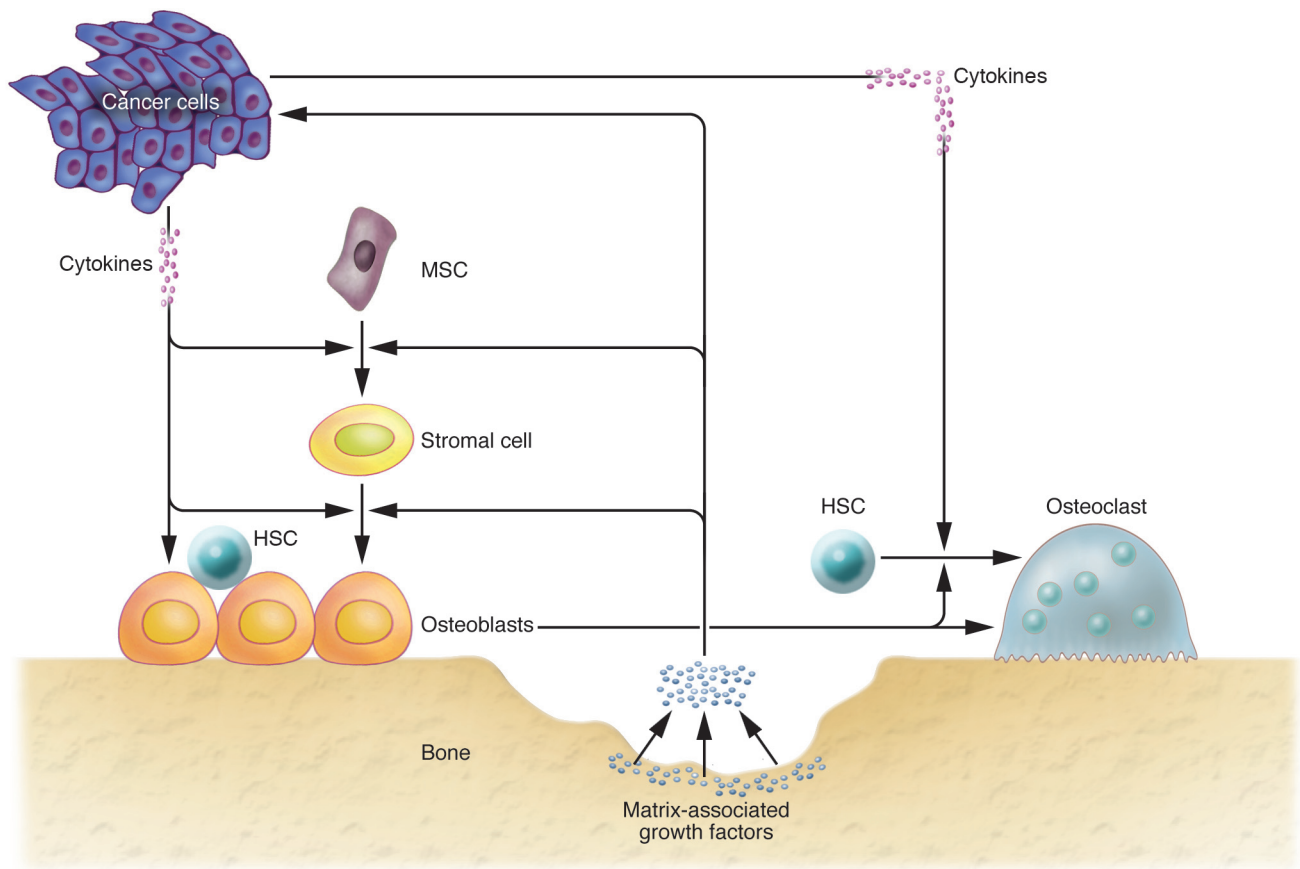
### Osteoblast biology

Osteoblasts are specialized fibroblasts that secrete and calcify a specific matrix. Their lineage specification from mesenchymal stem cells is regulated by a plethora of signals. A range of cytokines modulate osteoblast differentiation, including bone matrix-derived TGF- $\beta$ , bone morphogenic protein 2 (BMP-2), BMP-4, and BMP-7, and their inhibitors noggin, chordin, gremlin, and sclerostin, the last identified by positional cloning of families with increased bone mass. Similarly, numerous hormones impact osteoblast function positively including IGF-1, parathyroid hormone (PTH), PTH-related protein (PTHrP), 1,25(OH) $_2$ D $_3$ , leptin, glucocorticoids, the Notch pathway, and members of the leukemia inhibitory factor/IL-6 family.

Transcription factors that regulate the osteoblast include a range of homeodomain proteins: the activator protein (AP) family members Jun, Fos, and Fra, Smads, CCAAT/enhancer binding protein  $\beta$  (C/EBP $\beta$ ) and C/EBP $\delta$ , lymphoid-enhancing factor (a Wnt effector), activating transcription factor 4, Runt-related transcription factor 2 (Runx2), and osterix, the last 3 of which are considered master genes for osteoblast differentiation. In contrast to the osteoclast (see below), the osteoblast's best-characterized intracellular signaling pathway is the p42/44 MAPK system.

Osteoblasts ligate existing matrix via  $\beta_1$  integrins, forming a monolayer that is linked by cadherins. Once active, the cells secrete a matrix containing type I collagen and smaller but significant amounts of osteocalcin, matrix gla protein, osteopontin, bone sialoprotein, many minor components, and, importantly, growth factors such as BMPs and TGF- $\beta$ . Key ectoproteins, including progressive ankylosis gene (ANK) and tissue nonspecific alkaline phosphatase (TNAP), export pyrophosphate generated intracellularly and cleave this small-molecule inhibitor of calcification, respectively (34). In contrast to their proapoptotic role in osteoclasts, bisphosphonates increase osteoblast lifespan and perhaps function (35).

The text below focuses on Wnt signaling in osteoblasts and the role of these cells as supporters of the HSC niche and targets for osteoclastogenic hormones. Detailed reviews of osteoblast biology are available (36–40).



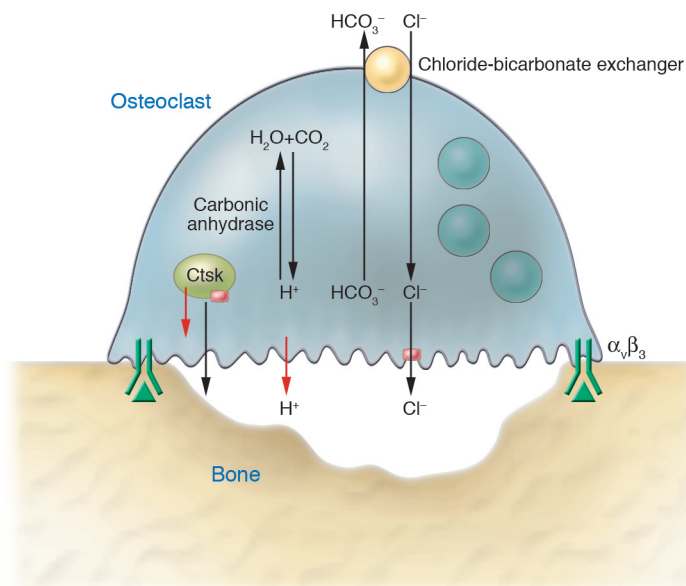
**Figure 2**

Cell-cell interactions in bone marrow. HSCs, the precursors of osteoclasts, reside in a stem cell niche provided by osteoblasts, which, together with stromal cells, derive from mesenchymal stem cells. Bone degradation (arrows) results in release of matrix-associated growth factors, which stimulate mesenchymal cells and thus bone formation. This “coupling” is an essential consequence of osteoclast activity (98). Additionally, matrix-derived factors stimulate cancer cell proliferation in the so-called “vicious cycle.” Finally, cancer cells release cytokines that target mesenchymal cells and thus activate bone resorption.

### Wnt signaling

In this series, the Review article by Ormond MacDougald and colleagues (41) focuses on the role of Wnt signaling in osteoblast formation and function. Wnts, a family of secreted glycoproteins with multiple inhibitors, are ligands for the family of 7-membrane-spanning frizzled receptors and play a prominent role in both the early and later stages of osteoblast differentiation. Wnt antagonists include Dickkopfs (Dkks) and secreted frizzled-related proteins (sFRPs). While their signaling pathways were characterized originally in terms of development, Wnts regulate numerous cellular functions and have been linked recently to cancer and stem cell biology. It is also clear that individual Wnts utilize canonical and noncanonical pathways (42); the endogenous Wnt ligands in bone remain unidentified, although Wnt10b was recently implicated by MacDougald et al. (43). The canonical pathway is well established and involves Wnt-dependent inhibition of proteasome-mediated degradation of  $\beta$ -catenin, which forms complexes with members of the T cell factor/lymphoid enhancer binding factor (TCF/LEF) family that regulate transcription when it accumulates in the nucleus. The noncanonical pathway, also frizzled dependent, activates different intracellular signals including the calcium-calmodulin-PKC axis and the Rho family of small GTPases.

MacDougald and colleagues review the current information on Wnts and bone biology. The story began with the identification of loss- and gain-of-function mutations in LDL receptor-related protein 5 (LRP5), a frizzled coreceptor, that correlate with changes in human bone mass. More recent studies showed that  $\beta$ -catenin, which is downstream of the Wnt-LRP5/6-frizzled axis, is indispensable for osteoblast differentiation in the mouse (44). Several papers cited by MacDougald et al. are worthy of comment here. Whereas mice lacking frizzled-related protein 1 have increased bone mass arising from markedly decreased osteoblast apoptosis, and *Lrp5*<sup>-/-</sup> mice exhibit a predominant deficit in osteoblast number and function, Col1-Cre;  $\beta$ -catenin<sup>c/c</sup> animals show mainly a secondary defect in osteoclasts. These results may reflect different modes of Wnt signaling: canonical versus noncanonical pathways, Wnt-dependent versus -independent signaling, or unique roles for different Wnt family members. Identification of downstream targets of Wnt signaling is an underexplored subject: osteoprotegerin (OPG) was identified as a direct target in osteoblasts, and the anabolic genes subject to Wnt regulation are not known but may include BMPs. Despite the implied importance of canonical Wnt signaling in osteoblast biology, the role of the TCF/LEF family of

**Figure 3**

Mechanism of osteoclastic bone resorption. The osteoclast adheres to bone via binding of RGD-containing proteins (green triangle) to the integrin  $\alpha_v\beta_3$ , initiating signals that lead to insertion into the plasma membrane of lysosomal vesicles that contain cathepsin K (Ctsk). Consequently, the cells generate a ruffled border above the resorption lacuna, into which is secreted hydrochloric acid and acidic proteases such as cathepsin K. The acid is generated by the combined actions of a vacuolar  $H^+$  ATPase (red arrow), its coupled  $Cl^-$  channel (pink box), and a basolateral chloride–bicarbonate exchanger. Carbonic anhydrase converts  $CO_2$  and  $H_2O$  into  $H^+$  and  $HCO_3^-$ . Solubilized mineral components are released when the cell migrates; organic degradation products are partially released similarly and partially transcytosed to the basolateral surface for release.

transcription factors is unclear. Since  $\beta$ -catenin participates in transcriptional complexes with molecules other than TCF/LEFs, some target genes may not be regulated via TCF/LEF binding sites. Finally, MacDougald et al. may understate the problem of targeting glycogen synthase kinase 3 (GSK3), a downstream effector of Wnt/ $\beta$ -catenin signaling and a molecule for which a number of potent inhibitors have been developed (45). As pointed out, long-term treatment with GSK3 inhibitors may predispose cells to an oncogenic mutation. On the other hand, lithium ions, a nonspecific inhibitor of GSK3, have been used for decades without apparent problems in this regard (45).

### The hematopoietic stem cell niche

In the Review by Tong Yin and Linheng Li (46), the authors summarize another new and exciting arena in bone biology, the HSC niche. The model, proposed simultaneously by Li and coworkers and Calvi et al. (47, 48) suggests that mesenchymal cells, in the form of bone-lining osteoblasts, are central to the maintenance of HSCs and their proliferation and/or differentiation. The system is complex since (a) many ligands and receptors that modulate interaction between myeloid and mesenchymal cells and hence expression of paracrine factors are present on both cell types and (b) many of the ligands and/or receptors are themselves regulated by cytokines. The authors also summarize data suggesting that bone marrow endothelium acts as a niche for a functionally distinct cell population. In related findings, the work of Brandi and Collin-Osdoby indicates that vascular endothelial cells respond to inflammatory cytokines by secreting receptor activator of NF- $\kappa$ B ligand (RANKL) and chemokines that are chemoattractive for osteoclast precursors and augment the osteoclastogenic capacity of RANKL (49).

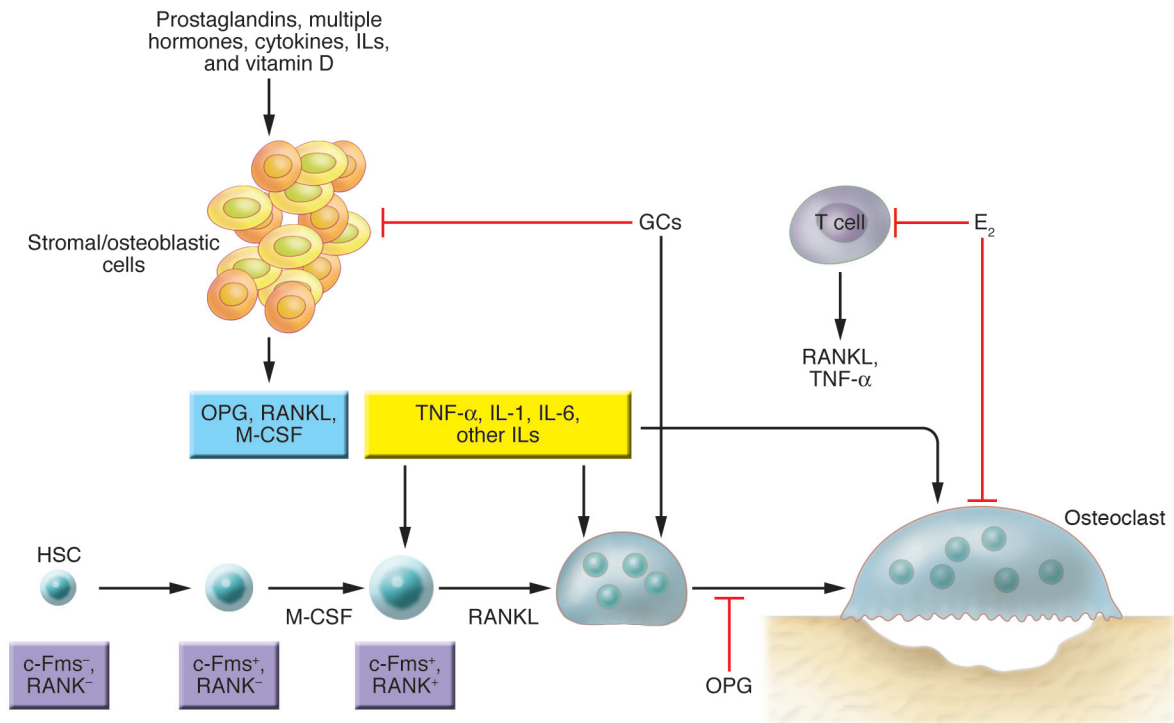
Bone marrow is also the environment in which specific metastases manifest their osteolytic and/or osteogenic phenotypes. Cancer cells facilitate their infiltration into the marrow cavity by stimulating osteoclast formation and function. An initial stimulus is PTHrP generation by lung and breast cancer cells (50–52), thus enhancing mesenchymal production of RANKL and M-CSF while decreasing that of OPG (see *Osteoclast biology*). The resulting

increase in matrix dissolution releases bone-residing cytokines and growth factors that, by feedback mechanisms, increase growth and/or survival of cancer cells. This loop has been termed “the vicious cycle” (Figure 2) (51). Multiple myeloma uses a different but related strategy, namely secretion of macrophage inhibitory protein 1 $\alpha$  (MIP-1 $\alpha$ ) and monocyte chemoattractant protein-1 (MCP-1), both of which are chemotactic and proliferative for osteoclast precursors (53, 54). Metastasis of prostate cancer to bone has both lytic and blastic components, with the net result being deposition of excess woven bone (55). A recent study suggests that BMP-6, produced by cancer cells, stimulates their invasive and proliferative capacity (56). Similarly, inhibition of VEGF also blunts prostate metastasis (57), suggesting that multiple cytokines play a role in proliferation and invasion of this cancer. Future studies will undoubtedly reveal additional molecules mediating bone loss in metastatic disease.

### Osteoclast biology

The osteoclast is generated by differentiation and fusion of precursors of the monocyte/macrophage lineage, giving rise to a polykaryon with unique cellular and molecular properties. Two cytokines mediate basal osteoclastogenesis, RANKL (58) and M-CSF, also called CSF-1 (59). Both proteins, produced by marrow stromal cells and their derivative osteoblasts, are membrane bound, and thus differentiation of osteoclasts requires direct interaction of these nonhematopoietic, bone-residing cells with osteoclast precursors (60). The discovery of RANKL was preceded by identification of its physiological inhibitor OPG, to which it binds with high affinity (61). M-CSF regulates many aspects of myeloid precursors and mature osteoclasts, including proliferation and/or survival, differentiation, and the cytoskeletal rearrangements required for efficient bone resorption (59).

A combination of biochemistry and genetics helps to explain how osteoclasts resorb bone (58, 62). The capacity of osteoclasts to isolate the enclosed area between themselves and the underlying bone is at the heart of their function. The acidic pH (~4.5) decalcifies the tissue, exposing the organic matrix to degradation by lysosomal-derived proteases, particularly cathepsin K (Figure 3).



**Figure 4**

Role of cytokines, hormones, steroids, and prostaglandins in osteoclast formation. Under the influence of other cytokines (not shown), an HSC commits to the myeloid lineage, expresses the M-CSF receptor colony-stimulating factor receptor 1 (c-Fms) and then, driven by M-CSF/c-Fms signaling and the RANKL receptor RANK, differentiates into an osteoclast. Mesenchymal cells in the marrow respond to a range of hormonal and cytokine stimuli, secreting a mixture of pro- and antiosteoclastogenic proteins, the latter primarily being OPG. Glucocorticoids (GCs) suppress bone resorption indirectly (by inducing death of osteoblasts) but possibly also target osteoclasts and/or their precursors. Estrogen (E<sub>2</sub>) inhibits secretion of RANKL and TNF- $\alpha$  by T cells via a complex mechanism (not shown); the sex steroid also inhibits osteoclast function.

The fact that dysfunction of the proton pump, Cl<sup>-</sup> channel, or cathepsin K results in human diseases of excess bone mass, namely osteopetrosis or pyknodysostosis (58, 62), attests to their critical role in osteoclast function.

This model of bone degradation requires close apposition between the osteoclast and bone, a role provided by integrins in the form of  $\alpha\beta$  heterodimers (63). Consistent with the fact that  $\alpha_v\beta_3$  is the principal integrin in osteoclasts,  $\beta_3^{-/-}$  mice are hypocalcemic and generate fewer and shallower resorptive lacunae on dentin slices than do their wild-type counterparts (62). Based on these and many in vitro observations, small-molecule inhibitors of osteoclast function that target  $\alpha_v\beta_3$  are in development (64).

Integrin activation mediates both cellular adhesion and transmembrane signaling (65). Important downstream transducers include the proto-oncogene c-src, important for membrane ruffling and osteoclast migration (66), and Rac and Rho, members of a small subfamily of the small GTPase superfamily that are central to remodeling of the actin cytoskeleton in many cell types (67) and play a similar role in osteoclastic bone resorption (68). It is now clear that bisphosphonates block bone resorption by inhibiting membrane targeting of a number of small GTPases (69).

### Regulators of osteoclast function

*Small molecules.* The steroid hormone 1,25(OH)<sub>2</sub>D<sub>3</sub> plays a major role in regulating calcium and phosphate homeostasis. Deficiency of the hormone increases bone loss by altering the RANKL/OPG

ratio secondary to hypocalcemia and resulting in hyperparathyroidism (61). In contrast, high levels of the steroid directly stimulate mesenchymal cell expression of RANKL and suppresses that of OPG (61) as well as suppress the proosteoclastogenic hormone PTH (70). A recent intriguing study identifies a vitamin D analog that prevents bone loss in oophorectomized mice by inhibiting RANKL-induced expression of c-Fos, a transcription factor required for osteoclastogenesis (71).

Both endogenous glucocorticoids and their synthetic analogs, which continue to be a mainstay of immunosuppressive therapy, have major impacts on bone biology (38) because of severe osteoporosis arising from decreased bone formation and resorption (low-turnover osteoporosis). The majority of the evidence focuses on the osteoblast as the prime target, with glucocorticoids increasing apoptosis of these bone-forming cells (72). However, human studies document a rapid initial decrease in bone resorption, suggesting that the osteoclast and/or its precursors may also be impacted by the steroid via an ill-defined mechanism. One possibility is that the apoptotic impact on osteoblasts decreases local levels of RANKL and M-CSF. Alternatively, glucocorticoids have been shown to decrease osteoclast apoptosis (73).

A wide range of clinical information demonstrates that excess prostaglandins stimulate bone loss by targeting stromal and osteoblastic cells, thus stimulating expression of RANKL and suppressing that of OPG (33). This increase in the RANKL/OPG ratio is sufficient in itself to explain the clinical findings of





increased osteoclastic activity. However, highlighting again the dilemma of interpreting *in vitro* studies, prostaglandins regulate osteoclastogenesis *per se* in murine cell cultures but have anabolic consequences *in vivo* (74).

**Proteins.** In addition to M-CSF and RANKL, several proteins play important roles in osteoclast biology (Figure 4). OPG, an endogenous RANKL inhibitor, is secreted by mesenchymal cells both basally and in response to other regulatory signals, including cytokines and bone-targeting steroids (61). Genetic deletion of OPG in mice and humans leads to profound osteopetrosis (58, 75), while global overexpression of the molecule in mice results in severe osteoporosis (58). Importantly, postmenopausal women exhibit higher levels of RANKL on their bone marrow stroma, and treatment with estrogen reverses this outcome (76). Finally, a recent study demonstrates that a humanized, monospecific antibody to RANKL increases bone loss in osteoporosis patients (77). Together, these observations indicate that circulating OPG modulates the bone-resorptive activity of RANKL and suggest that the RANKL/OPG ratio in serum will become a clinically important index.

Proinflammatory cytokines suppress OPG expression while enhancing that of RANKL (61). Thus many patients with osteolytic disease have altered serum levels of RANKL and/or OPG as a result of high levels of TNF- $\alpha$ , IL-1, PTH, or PTHrP. Serum PTH levels are increased in hyperparathyroidism of whatever etiology, whereas PTHrP is secreted by bone-targeting lung and breast carcinomas (50, 51). Attesting to the importance of TNF- $\alpha$ , antibodies against the cytokine or a soluble TNF receptor-IgG fusion protein potently suppress bone loss in disorders of inflammatory osteolysis such as rheumatoid arthritis (78). In humans, an IgG fusion protein containing the active component of the IL-1 receptor antagonist enhances the ability of anti-TNF- $\alpha$  antibodies to decrease bone loss in rheumatoid arthritis (79).

## Osteoimmunology

It has become clear over the past few years that the immune system plays an important role in bone biology; perhaps nowhere is this more evident than in the work of the Pacifici group. In this series, the Review by M. Neale Weitzmann and Roberto Pacifici explores how estrogen deprivation leads to bone loss (80). In short, they report that estrogen, the main sex steroid regulating bone mass in both men and women (81), suppresses the capacity of antigen-presenting cells to stimulate T cell activation, an event that normally results in secretion of a range of proresorptive cytokines including TNF- $\alpha$ , IL-6, and RANKL. Notably, in this circumstance RANKL is cleaved from the cell surface. TNF- $\alpha$  and IL-6 act on stromal cells and osteoclast precursors to enhance bone resorption by regulating expression of pro- (i.e., RANKL and M-CSF) and antiosteoclastogenic (i.e., OPG) cytokines in the case of mesenchymal cells and by synergizing with RANKL itself in the case of myeloid osteoclast precursors.

While these studies underscore the interface between bone and the immune system, they represent only a fraction of the work in the “hot” new area of osteoimmunology. The elegant work of Takayanagi and colleagues suggests that IFN- $\gamma$  is an important suppressor of osteoclast formation and function (82). Nevertheless, these findings are in conflict with *in vivo* observations in humans, including the report that IFN- $\gamma$  treatment of children with osteopetrosis ameliorates the disease (83) and the fact that a number of *in vivo* studies indicate that IFN- $\gamma$  stimulates bone resorption (ref. 84 and references therein). This conundrum

highlights again the need to discriminate between *in vitro* culture experiments using single cytokines and results *in vivo*. Many additional reports have implicated other cytokines in regulation of the osteoclast, including numerous ILs, GM-CSF, IFN- $\gamma$ , stromal cell-derived factor 1, MIP-1 $\alpha$ , and MCP (85–88), but at this time the results are either contradictory, as for GM-CSF in the murine versus human systems, or lack direct proof in humans. Future studies are likely to clarify the currently confusing data set. Finally, several groups have provided evidence that molecules traditionally considered as solely immune receptors, such as DNAX activating protein of 12 kDa (DAP12), FcR $\gamma$  and triggering receptors expressed in myeloid cells, and their ligands on cells of the stromal and myeloid/lymphoid lineages, are downstream of RANKL and M-CSF signaling (89, 90). Mutations in DAP12 lead to a rare bone disease, Nasu-Hakola disease, in which patients exhibit bone cysts and concomitant demyelination (91).

## More than skin deep, and down to the bone

Finally, we return to the question posed in the introduction. What are the common links between skin and bone? One recurring theme is that Wnts are important and exhibit diversity in function in both tissues. In the skin, Wnts, BMPs, and other signaling pathways are required for early patterning and for morphogenesis of the skin appendages including hair, teeth, nails, and mammary glands (28, 29). Likewise, in bone, Wnts, BMPs, and FGFs are required, among other factors, for critical aspects of bone development. In early development, the overlying ectoderm plays an instructive role and secretes morphogens to the underlying bone in the form of the apical ectodermal ridge during limb outgrowth (92, 93). In the adult, Wnt signaling is reprised again during tissue homeostasis and is a key regulator of the hair cycle as well as osteoblast and osteoclast regulation. Both skin and bone are home to specialized stem cell niches that provide a safe haven for their relevant progenitor cell populations. There are common transcriptional regulators of bone and skin, such as the vitamin D receptor, whose cascade of downstream effectors are clearly crucial in both tissues, since mutations in this gene cause profound effects in the form of irreversible alopecia as well as rickets (94). It has already been shown that hair follicle dermal papilla cells can differentiate into adipocyte and osteogenic cells in culture (95), and further, that they can repopulate the entire hematopoietic system (96). The dermal papilla is easily identified within the skin by its strong expression of alkaline phosphatase, generally considered a marker of bone formation (97). Given the anatomical proximity of the two tissues, it is not beyond the realm of possibility that a common adult mesenchymal progenitor cell population supports both the skin dermis and underlying bone. Despite their phenotypic extremes of softness and hardness, future studies in skin and bone biology may reveal that they have far more in common than meets the eye.

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## review series introduction

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