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Lecture Notes: Bone Biochemistry and Regeneration

Bones are the organs of the skeletal system. Functions of the skeletal system are: support - it forms the body's framework to support the muscles and organs.

protection - the skeletal systems protects by

1) forming the bony cavities around organs, e.g. the thoracic cavity protects the heart and lungs, the cranial cavity protects the brain.

2) the red marrow in bones produces white blood cells which protect against invading microorganisms.

movement - bones form joints which provide levers for movement such as walking, lifting, etc.

hematopoiesis (blood cell production) - the red marrow produces both red and white blood cells.

mineral storage and homeostasis - the skeleton forms a reservoir of minerals, especially calcium, for maintenance of homeostasis.

Cell types

Osteoprogenitor cells - these are like "stem cells" for bone. They have the capacity to divide and proliferate to form osteoblasts, bone forming cells which actively produce bone tissue. They comprise the periosteal and endosteal cells (see below) which line the bone and its cavities. In mature bone where growth and remodeling is not occurring these cells are quiescent, but they are thought to function in maintenance and nutritional support of the osteocytes in the underlying matrix, with which they connect by means of gap junctions.

Osteoprogenitor cells are derived from mesenchymal cells (a fundamental embryological germ tissue) and have the ability to differentiate into adipose cells,

chondroblasts, and fibroblasts and can modify their morphologic (physical) and physiologic characteristics in response to specific stimuli.

Osteoblasts - these are the "bone forming cells" which secrete the collagen and ground substance that constitutes unmineralized bone (osteoid), and subsequently are responsible for calcification of the matrix. These cells also communicate with one another and with osteocytes by gap junctions.

Osteocytes are mature bone cells, differentiated from osteoblasts, which are responsible for maintaining the bone matrix. They can synthesize and resorb (break down) the matrix to maintain homeostasis. Each osteocyte occupies a space, the lacuna, which conforms to the shape of the cell surrounded by matrix secreted when the cell was an osteoblast. Osteocytes extend processes through canaliculi to connect to neighboring cells by means of gap junctions.

Osteoclasts are large multinucleated cells whose function is to resorb bone. Osteoclasts dissolve the matrix and osteoid with acids and hydrolytic enzymes. Osteoclasts are phagocytic and are derived from monocytes and not from the same line as the other bone cells.

Bone Formation and Remodeling

Mature bone is a continuously remodeling tissue in which resorption and formation take place sequentially throughout life. The process whereby this sequential coordination between osteoclast-mediated resorption and osteoblast-mediated formation is maintained is termed coupling. The coupling of osteoblast and osteoclast function is brought about by a number of mechanisms. Possibly the most important is the fact that the major hormones (PTH and vitamin D) and local factors (IL-1) that stimulate bone resorption do not have their receptors on the osteoclasts but instead on the bone lining cells. Thus the signal for bone resorption by the osteoclast is mediated by the osteoblast cell lineage. This signalling involves the release of soluble factors produced by the bone lining cells (M-CSF and osteoprotegerin) and the interaction of cell surface molecules on the bone lining cells (TRANCE) and recruited osteoclasts (RANK). In addition, the osteoclasts will not bind to bone surfaces covered by unmineralized osteoid, but first require the bone lining cells to degrade the osteoid to expose underlying mineral. Demineralization of bone by the osteoclast releases matrix constituents (such as BMP and TGF β) that can stimulate the differentiation and proliferation of osteoblast progenitors or stimulate matrix production by the osteoblasts, and so initiate new bone formation. Osteoclast action is terminated by apoptosis (programmed cell death) and the prevention of new recruitment by osteoprotegerin production by the osteoblasts.

Some factors that play a role in bone formation and remodeling

Bone Morphogenetic Proteins (BMPs)

BMPs are a group of growth factors also known as cytokines, which are pivotal in morphogenic signaling. In bone, they initiate signaling for bone formation, by activation of pathways that lead to the generation of osteoblasts for bone formation. Several commercial efforts are directed at developing BMPs for applications in bone repair and regeneration. BMPs induce cell migration, proliferation, and differentiation, and it is not entirely clear yet which of these processes dominate in vivo. In order for BMPs to be effective, progenitor cells that can be induced to form bone must be present in the local area. This means that BMPs will not likely be effective in very large defects, or defects which has been compromised by irradiation or infection.

Parathyroid hormone

Plasma calcium levels are regulated by parathyroid hormone (PTH), with increased production of the hormone being stimulated by low calcium levels and decreased production by high calcium levels. The major function of parathyroid hormone is to increase serum calcium. It does this 1) by stimulating osteoclastic bone resorption, 2) by preventing calcium excretion by the kidneys, and 3) by stimulating the conversion of vitamin D to the active metabolite responsible for enhancing calcium absorption through the intestines. PTH stimulates bone resorption by promoting osteoclast formation, promoting ruffled border formation, and promoting lysosomal enzyme production and carbonic anhydrase. This stimulation is, however, indirect as the receptors for PTH reside on the osteoblast not on the osteoclast. A related protein, PTHrP, is produced by some tumors. It can interact with the PTH receptors and produce the hypercalcemia associated with malignancy.

Vitamin D

Vitamin D is not itself hormonally active, but requires hydroxylation to 1,25-dihydroxyvitamin D (calcitriol). The first metabolic modification is 25-hydroxylation occurring in the liver, followed subsequently by 1-hydroxylation in the kidney. Both steps are catalyzed by specific hydroxylases. Activity of the 1-hydroxylase in the kidney is enhanced by the action of PTH and low calcium levels. The active metabolite can both promote bone mineral formation through stimulating calcium and phosphate availability, and participate directly in bone resorption through osteoclast activation. As with PTH, the calcitriol receptors are possessed by the osteoblast not the osteoclast.

Clinical problems requiring replacement

Regenerative medicine

Regenerative medicine or **stem cell therapy** is the "process of replacing or regenerating human cells, tissues or organs to restore or establish normal function".

Stem cells are biological cells found in all multicellular organisms, that can divide (mitosis) and differentiate into diverse specialized cell types and can self-renew to produce more stem cells.

Stem cells are capable of dividing and renewing themselves for long periods. Unlike muscle cells, blood cells, or nerve cells—which do not normally replicate themselves—stem cells may replicate many times, or proliferate. A starting population of stem cells that proliferates for many months in the laboratory can yield millions of cells. If the resulting cells continue to be unspecialized, like the parent stem cells, the cells are said to be capable of long-term self-renewal.

Types of stem cells

Embryonic Stem Cells (ESCs) are unspecialized cells that can give rise to specialized cells. They are capable of dividing and self renewing for long periods under appropriate conditions. These cells are typically taken from blastocysts that are not implanted for pregnancy (invitro fertilization). They are usually frozen for future use by the couple who produced them, or are ultimately discarded.

Some disadvantages to ESCs are

- (1) Hard to control: They may pass through several intermediate stages before becoming the cell type needed to treat a particular disease.
- (2) Rejected by the immune system: The immune profile of the specialized cells would differ from that of the recipient.
- (3) Ethically controversial: Many who believe life begins at conception say that the informed consent by patient donors does not remove the ethical stigma of doing research on human embryos.

Advantages: Virtually unlimited pluripotent potential

Adult stem cells have been identified in many organs and tissues, including brain, bone marrow, peripheral blood, blood vessels, skeletal muscle, skin, teeth, heart, gut, liver, ovarian epithelium, and testis. They are thought to reside in a specific area of each tissue (called a "stem cell niche").

Stem cells may remain quiescent (non-dividing) for long periods of time until they are activated by a normal need for more cells to maintain tissues, or by disease or tissue injury.

Some strategies discussed in class

Gene Therapy (in presentation slides)
Cell Based - Tissue Engineering

Current clinical application: Platelet Rich Plasma + BMP

Platelet Rich Plasma (PRP) is a substance that is a by-product of blood (plasma) that is rich in platelets. Up until recently, its use has been solely confined to use in hospitals. This was due to the cost of separating the platelets from the blood and the large amount of blood needed to produce an appropriate amount of platelets. New technology allows the doctor to harvest and produce an adequate amount of platelets from only 20 cc of blood drawn from the patient while they are having outpatient surgery.

PRP allows the body to take full advantage of the normal healing pathways at a greatly accelerated rate. While your body goes through the healing process, it rushes numerous cells and cell-types to the wound in order to initiate the correct healing process. One of those cell types are the platelets. Platelets have several functions, including the release of growth factors (GF) into the wound and the formation of blood clots. These GF (platelet derived growth factors PDGF, transforming growth factor beta TGF, and insulin-like growth factor ILGF) function to help the body in repairing itself by stimulating the stem cells to regenerate new tissue. The more growth factors that are released into the wound, the more stem cells that are stimulated to produce new host tissue. Therefore, it's obvious that PRP allows the body to heal quickly and efficiently.

A subfamily of TGF, is bone morphogenic protein (BMP, above). As you know, BMP has been proven to encourage the formation of new bone in research studies that have been done on animals and humans. The results of these research studies is greatly significant to the oral surgeon who places dental implants. By adding PRP, and thus BMP, to the implant sites that have been substituted with bone particles, the implant surgeon can now grow bone more quickly and predictably.