Avascular Necrosis of the Femoral Head: Are Any Genes Involved?

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Abstract
Avascular necrosis of the femoral head (ANFH) is a pathologic process that results from interruption of blood supply to the femur bone resulting in the death of bone cells and collapse of the femoral head. Nontraumatic ANFH continues to be a significant challenge to orthopedic surgeons. While the exact mechanisms remain elusive, many new insights have emerged from research in the last decade that has given us a clearer picture of the pathogenesis of nontraumatic ANFH. Progression to the end stage of ANFH appears to be related to five main mechanisms: hypercoagulable conditions, angiogenesis suppressions, hyperadipogenesis, heritable states, and switching the bone remodelling into bone resorption. Researchers have been examining the pathogenic mechanisms of ANFH but none of these theories have been firmly confirmed although some appear more plausible than others. All of these factors can switch bone remodelling into bone resorption, which can further lead to ANFH progression ending up to femoral head collapse.

Key words: Avascular, Avascular necrosis, Femur, Osteonecrosis

Introduction
Avascular necrosis of the femoral head (ANFH) is manifested with death of bone cells resulting in the impairment of normal reparative processes within the microfractures in the femoral head (1). Because the exact pathophysiology is not elucidated yet, there is a variable nomenclature for this condition. The name avascular necrosis (also known as osteonecrosis, aseptic, or ischemic necrosis) may be misleading as it has not been demonstrated that bone cells die because of necrosis. In the other words, characteristics of necrosis in the soft tissue including cell swelling and inflammatory responses do not occur in ANFH (2).

Its true prevalence of the disease is unknown. Approximately 10,000 to 20,000 new patients with ANFH are diagnosed each year in the United States and there are 300,000-600,000 people having the disease (3). Almost 75% of patients with ANFH are between 30 to 60 years of age (4). Since most patients with the disease are at the peak of their productive years, there is a considerable effect on the workforce and subsequently on the economics. The understanding of its pathophysiology and its progression will help us prevent patients, their family and society from these consequences.

A number of studies have examined the pathogenic mechanisms of avascular necrosis in the femoral head and none of the current hypotheses have been firmly established although some appear more plausible than others. In this review, different molecular mechanisms and several mediators important in pathogenesis of ANFH are going to be discussed.

Coronary disruption of the hip
Role of Endothelium in Avascular Necrosis

Although the pathophysiology of non-traumatic ANFH remains unclear, most scholars agree that ANFH is associated with thrombus formation in the microvasculature of the femoral head as depicted in the vascular hypothesis for ANFH (1). It appears that damage to the endothelial cell membrane combined in some cases with an increased propensity to blood clot formation, is the key causing blood flow interruption. The endothelial cell monolayer constitutes the inner...
lining of the vascular wall and plays an essential role in the homeostasis of the blood vessel. Due to its unique localization, the endothelium is continuously exposed to inflammatory cells and circulating factors, which could induce endothelial activation and/or endothelial injury (5). In a study by Jacobs et al. damage or abnormality of the reticular vessels was suggested to be the underlying mechanism of ANFH (6). Damage to the endothelial cells may result in abnormal blood coagulation and thrombi formation. Thus, avascular necrosis could occur distal to the site of arterial occlusion. Slichter et al. postulated that endothelial cell damage is followed by platelet thrombus formation with secondary fibrin deposition in the femoral head in dysbaric osteonecrosis (7). Li et al. also showed endothelial cell damage as well as a high coagulant and a low fibrinolytic milieu the possible pathologic mechanisms of glucocorticoid-induced ANFH (8). It is noteworthy that in a recent study, alpha-2-macroglobulin (A2M) gene expression was the most significantly upregulated gene in a glucocorticoid-induced ANFH rat model. In this model, glucocorticoids (GCs) seem to modulate significantly the A2M levels (9). GCs can change the A2M gene expression by attaching to a glucocorticoid response element (GRE), a conserved consensus sequence for a putative GC receptor DNA binding site, in the 5'-flanking region proximal to the A2M gene promoter (10). Several other literatures also support the role of GCs in the modulation of A2M (11, 12). A2M has been identified on the luminal surface of endothelial cells in sections of normal human arteries and veins (13). It has also been implicated in hemostasis as a regulator of thrombin and in the development of thromboembolism in children (14-17).

**Avascular Necrosis and the Coagulation Pathway**
Glucocorticoids are the most common non-traumatic cause of ANFH as between 5% to 40% of patients treated with long-term GCs develop ANFH (3, 18). Furthermore, with regards to the regulatory effect of GCs on procoagulation mediators (Factor VIII & IX & VWF) and fibrinolysis inhibitor mediator (PAI-1), there is a possible role of GCs on coagulation (19-21).

Eduardo Ramacciotti et al. in 2010 introduced Alpha-2-Macroglobulin (A2M) as a protein influencing on thrombosis through inflammation, cell shedding, inhibition of fibrinolysis, and hemostatic plug formation. On the other hand, it could play an important role in thrombogenesis and fibrinolysis. It works as an inhibitor of fibrinolysis by inhibiting plasmin and kallikrein, and as an inhibitor of coagulation by inhibiting thrombin (22). Thus, it can be concluded that GCs can make changes in endothelial function both by modulation in A2M gene expression and through thrombosis formation that subsequently induces ischemia [Figure 1].

Björkman et al. in two separate studies in 2004 and 2005 suggested that the coagulation abnormalities in the form of factor V Leiden and prothrombin 20210A gene mutation might play a role in avascular necrosis of the knee and hip (23, 24). Zalavras et al. in 2004 also studied the thrombophilic factor V G1691A mutation (factor V Leiden) and G20210A prothrombin mutation in ANFH patients and introduced factor V Leiden, a genetic risk factor for venous thrombosis. This study and similar studies support the hypothesis that intravascular coagulation is a major pathogenetic mechanism of the disease (25).

**Ischemia in Avascular Necrosis**
Femoral head avascular necrosis can occur due to blood supply disruption, which results in hypoxic injury to the femoral head. Hypoxia-inducible factor-1 α (HIF-1α) is a master regulator of cellular response to hypoxia. It has been reported that there is a coordinated upregulation of HIF-1α and Vascular Endothelial Growth Factor (VEGF) expression under hypoxia in both RNA and protein levels after an ischemic event in the cartilage (26). Furthermore, VEGF expression was reduced by 44% after HIF-1α knockdown by siRNA transfection in conditional...

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**Figure 1. Different mechanisms of glucocorticoid effect on ANFH.**
HIF-1α knock out mice. VEGF is a well characterized angiogenic factor that is activated by hypoxia. All of these observations demonstrated that upregulation of VEGF in chondrocytes following femoral head ischemia is mediated partially through HIF-1α. These results are suggesting more vessel formation after inducing ischemia in pig femoral head via VEGF upregulated partially through HIF-1α (27). These findings introduce VEGF as a factor responsible for neo-vascularization and repair. Ding et al. in 2013 showed that transplantation of HIF-1α transgenic bone marrow cells (BMCs) that upregulated VEGF and enhanced both angiogenesis and osteogenesis potentially promotes the repair of necrotic area of GCs-induced ANFH (28).

Considering the results, new therapeutic modalities that can stimulate the repair process and restore normal vascular and growth after ischemic necrosis have been developed (29).

**Angiogenesis in Avascular Necrosis**

In ANFH, interruption of angiogenesis is a pathological process that may lead to impairment of the nutrient supply, cell death, and collapse of the bone. However, the process of angiogenesis in ANFH is not well understood (30). Recent studies have revealed a strong correlation between ANFH and the expression of VEGF, a major inducer of angiogenesis and more importantly in bone formation and repair (31-35). VEGF regulates bone remodelling by attracting endothelial cells and osteoclasts as well as stimulating osteoblast differentiation (36). Other studies also support augmentation of angiogenesis in bone tissue by VEGF gene transfection, which accelerates bone repairing (37, 38). All of the above besides the similar studies suggest that VEGF has a potential therapeutic effect for avascular necrosis of the bone tissue (39-41). Yang et al. in 2004, co-transfected bFGF and collagen genes and implanted it in a necrotic femoral head of an animal model and showed that angiogenesis in the bone tissue improved. Also they found that the repair process in the necrotic femoral head accelerated significantly (42). It has also been shown that nitric oxide regulates bone turn over through osteoblasts and osteoclasts. Nitric oxide production is impaired by the T-786C eNOS single nucleotide polymorphism. This leads to vasoconstriction, platelet aggregation, reduced angiogenesis, and reduced bone formation, all of which may be associated with avascular necrosis of the hip. In a study by Gludeck et al. in 2007, the association of T-786C eNOS polymorphism and resultant reduction of nitric oxide production was shown to contribute in the pathogenesis of idiopathic ANFH (43).

**Lipid Biosynthesis in Avascular Necrosis**

Many studies suggest that several factors are important in the etiology of ANFH, including GCs usage, alcoholism, infections, coagulation defects, and some autoimmune disorders. However, etiological and pathological mechanisms of ANFH have not been yet thoroughly investigated. As mentioned earlier, vascular hypothesis is considered to be most persuasive mechanisms among others to date (1). Several studies have identified that hyperlipidemia in the femoral head induced by GCs and alcohol use is associated with ANFH.

Kim et al. in 2008 evaluated the association between sterol regulatory element binding factor (SREBP-2) gene polymorphisms and the susceptibility of ANFH in the Korean population. SREBPs, which belong to the basic helix-loop-helix family of transcription factors, are important in lipogenesis, adipocyte development, and cholesterol homeostasis (44). Also in 2009 Lee et al. reported a polymorphism in intron 7 of the SREBP-1 gene associated with an increased risk of ANFH (45). This would provide the evidence of an association between ANFH and lipid metabolism.

In a study by Okazaki et al. in 2009, It has been shown that GCs-induced ANFH could be caused by disruption of the immune system via LPS-activated toll-like receptor 4 (TLR4) signaling pathway associated with a disruption of the innate immune system and lipid synthesis or metabolism in a rat model (46).

**Apoptosis in Avascular Necrosis**

The osteocyte is the most numerous and longest-living cell in bone. They have an active role in regulating bone homeostasis. For example, they can act as an orchestrator of bone remodeling through regulation of osteoblasts and osteoclasts. It has been reported that increased numbers of osteocytes undergo apoptosis in glucocorticoid- and alcohol-induced avascular necrosis (47-49). GCs and alcohol could have direct toxic effects on bone cells, and cause them to become apoptotic (50). The exact mechanisms of non-traumatic avascular necrosis remain elusive; many studies in the past decade have shown that the pathogenesis involves different pathways and factors (51). It was found that the expression of OPG, RANK, and RANKL was higher in the necrotic part than in the normal region in osteonecrotic samples (52). Another study showed different expression levels of bone morphogenetic protein (BMP) in the normal and necrotic sites of femoral heads in patients with avascular necrosis (53). Jun et al. in 2014 reported that the expression level of inducible nitric oxide synthase (iNOS) increased in osteonecrotic samples in compare to control samples suggesting that more nitric oxide was being produced in osteonecrotic samples. They also found that the apoptosis of numerous osteocytes in the avascular necrosis group was mainly associated with the consistently high expression level of iNOS. Furthermore, they supposed that using aminoguanidine, an inhibitor of iNOS, can reduce the production of iNOS in osteocytes and thus cause a reduction in apoptosis of osteocytes. They demonstrated that inhibition of iNOS could prevent non-traumatic avascular necrosis in an ANFH animal model (54). However, the apoptosis signalling pathway is not only mediated through the mitochondrion, but also the extracellular signals can activate caspase through Fas/CD95, and this pathway may play a significant role in the apoptosis of osteocytes in non-traumatic ANFH (55).
Bone Remodelling in Avascular Necrosis

Osteoprotegerin (OPG), Receptor Activator of Nuclear Factor kappa-B (RANK) and RANK ligand (RANKL) regulate the balance between osteoclasts and osteoblasts. The expression of these genes affects the maturation and function of osteoblasts and osteoclasts and bone remodelling (56). Samara et al. in 2014 suggested different expression mechanisms for OPG, RANK and RANKL. They could play an important role in the progress of bone remodelling in the necrotic area (52).

Bone morphogenetic proteins (BMPs) are other key proteins in regulating bone remodelling and healing (53). Implantation of osteogenic BMPs such as BMP-2 and BMP-7 at an osseous or extraosseous site results in bone and cartilage formation. These BMPs act primarily as differentiation factors, turning responsive mesenchymal cells into cartilage- and bone-forming cells (57). Implantation of human BMP-2 gene transfected bone marrow stem cells (BMSCs) can repair early-stage experimental femoral head necrosis (41). Thus, these osteogenic BMPs could be used as therapeutic targets for ANFH treatment.

Figure 2. Normal balance between bone formation and bone resorption resulting in bone tissue integrity (A). Bone remodeling balance switch into bone resorption, resulting in loss of femoral head collapse that leads to ANFH (B).

Inherited Avascular Necrosis

Although most cases of ANFH are sporadic, some idiopathic familial cases have been reported so far with some families having multiple affected members. Although genetic factors have been implicated in the etiology of ANFH, the causal gene has not been identified yet (58). COL2A1 mutations have been reported to be associated with ANFH particularly in bilateral cases in an autosomal dominant inheritance pathway in a Japanese family. The mutation (p.G1170S) leads to an amino acid change that perturbs a Gly-X-Y triple-helix repeat, which is fundamental structure in type II collagen. It has also been reported that abnormal large-diameter collagen fibrils in the epiphyseal cartilage of ANFH patients was present (59). This implies that abnormal type II collagen could be the cause of inherited ANFH (58). In another study, in 2008, Peiqiang et al. reported the same mutation of COL2A1 responsible for pathology confined to the hip joint, which presents as an isolated precocious hip osteoarthritis, ANFH, and Legg-Calve-Perthes disease in an age at onset dependent manner.

Bone remodelling involves the removal of mineralized bone by osteoclasts followed by the formation of bone matrix by the osteoblasts that subsequently become mineralized. Recent studies have showed that a balance in bone remodelling is maintained in the microenvironment of the femoral head. The regulation of bone remodelling is both local and systemic. The major systemic regulators include growth hormone, parathyroid hormone, glucocorticoids, thyroid hormones, and sex hormones. As far as local regulation of bone remodelling is concerned, many cytokines and growth factors including OPG/RANK/RANKL that affect bone cell functions have been recently identified (60). The balance between bone resorption and bone formation can maintain the integrity of the bone microenvironment. The regulators switch the balance into increasing bone resorption and decreasing bone formation in the bone microenvironment of femoral head, thus, femoral head will collapse [Figure2]. All of
the mechanisms previously described can lead to ANFH by switching the balance into bone resorption. Further investigations assessing the crossroad of the main molecular pathways are recommended.

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