Pathways of Skeletal Muscle Atrophy: HIV as a Model System?

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Skeletal Muscle Atrophy (SMA) is a phenomenon found in many diseases and disorders. SMA is characterized by protein degradation induced by various pathways. Ten years ago, little was known about the mechanisms that lead from these disorders to protein degradation. Current research focuses on the mechanisms thought to induce SMA. It is now known that many of these pathways involve ubiquitin conjugate accumulation and increased proteasome activity resulting in rapid protein degradation and decreased synthesis. HIV associated proteins, such as Vpr, cause overexpression of atrogin-1 which promotes atrophy. Cachexia operates mainly through the IKK/NF_B pathway and MuRF-1 Ub-ligase overexpression causes SMA. In contrast, the onset of oxidative stress increases intracellular calcium levels, activating endoproteases and stimulating myofilament degradation. Lastly, diabetes acts in a similar way. Low insulin levels trigger ub-conjugation and proteasome activity leading to SMA. In order to treat SMA in the aforementioned disorders, specified inhibitor drugs are being considered for hindering the pathway. This review proposes to use the HIV virus as a model to investigate how these diseases induce SMA with further investigations of the mechanisms from HIV to the aforementioned diseases. Possible treatments are associated with the reverse pathway, hypertrophy, which suppresses protein degradation and increases synthesis.

Introduction

Skeletal Muscle Atrophy (SMA) is a phenomenon common in many diseases and disorders such as starvation, disuse, denervation, sepsis, acidosis, cancer, diabetes, HIV/AIDS, oxidative stress, and more (Goldberg et al., 2004).

Ten years ago, it was assumed that these illnesses were related because they have many symptoms in common (Goldberg et al., 1986). Until recently, there had only been correlations between the appearance of SMA with these disorders and diseases but no direct evidence of their correlation existed.

SMA is characterized as the rapid degradation of proteins in voluntary muscles. With SMA, the capacity of the muscles to function is diminished. The mechanisms for this were previously unknown. For example, it was thought that atrophy during fasting was somehow caused primarily by enzymes and intracellular calcium uptake, though it was neither definitive nor specific (Russell et al., 1984).

Because SMA is associated with many different disorders, it is possible that there are many different pathways leading to protein degradation. How many are there? What is the nature of these pathways? Which diseases, if any, follow the same pathway? What specifically happens in these pathways? Though it has been shown that these pathways have an effect on SMA, there is still much that is not understood.

This study focuses on four different disorders that follow different pathways to SMA: HIV/AIDS, cachexia, oxidative stress, and diabetes. Each of these illnesses is highly studied and much information has been discovered in the last decade.

HIV/AIDS

HIV/AIDS has an exponential rate of infection concentrated largely in African women and children. There are no cures for HIV/AIDS and many aspects of the disease are still a mystery.

In the early 1990's, studies showed that one of the earliest clinical symptoms of HIV was weight loss and muscle wasting. When comparing muscle mass of a healthy person to that of an HIV/AIDS patient, the muscle mass of the HIV/AIDS patient was significantly lower. This was the first evidence that SMA is associated with HIV/AIDS (Gheradi et al., 1992).

Cachexia

Cachexia is a symptom of cancer known as a wasting syndrome characterized by the loss of adipose and muscle tissues. Approximately half of all cancer patients are afflicted with cachexia. In carcinomas of the pancreas and stomach, patients have an 80% chance of developing cachexia. Women with breast cancer have a 40% chance of developing this disorder (Iida et al., 1994).

Cachetic cancer patients experience at least a 10% decrease in body weight and usually die within six months of developing the disorder (Iida et al., 1994). Cachexia is one of the most significant causes of mortality in cancer.

Oxidatvie Stress

Oxidative stress is the general term for the level of oxidative damage in a cell. The oxidative damage is caused by reactive oxygen species, such as free radicals and peroxides that can affect a specific molecule or the entire organism. These species are usually by-products of other metabolic functions within a cell.

A cell is very sensitive to its redox status because the presence of oxidative stress increases intracellular calcium levels. Goldberg, et al. (1986) demonstrated that SMA was correlated with these higher calcium levels.

Diabetes Mellitus

Diabetes mellitus is a disease connected to insulin levels in the body. When insulin is not manufactured properly or at all, diabetes develops.

Type 1 diabetes is induced in children who consume excessive fats and sugars with little to no exercise. Type 2 diabetes manifests in middle-aged people due to insulin resistance. This type of diabetes

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is thought to be influence more by genetics than Type 1.

Studies have shown that insulin action was normal in nondiabetics. In diabetics, however, the level of insulin action in muscle cells was decreased. This was correlated with both a loss of muscle tissue and a build up of adipose tissue (Kelley et al., 1993).

All four of these disorders have been connected to SMA. We will show how these disorders lead to protein degradation and how, if at all, they are linked to each other. (Figure 1)

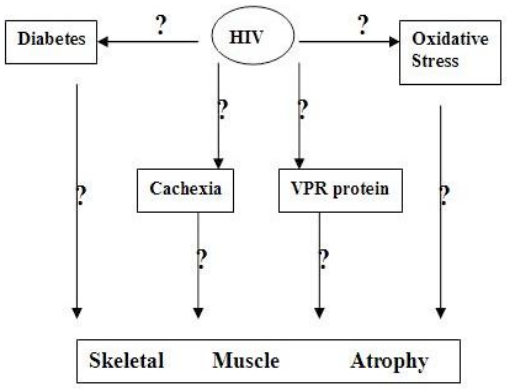

Figure 1. Correlation from HIV to diabetes, cachexia, oxidative stress.
Mechanisms to SMA are unknown

HIV and SMA

The most direct pathway from HIV to SMA is through the HIV Type-1 accessory protein, Vpr. Vpr is one of six accessory proteins produced in the body after the HIV genomic information is integrated into the host genome of a cell. It has several functions as a modulator of the host cell's activity. The most specific of these functions is that of nuclear translocation of the HIV-1 pre-integration complex. However, as part of its role in modulating the host cell, Vpr arrests the cell at the G2/M phase. This occurs when Vpr is present and binds to 14-3-3 proteins to change binding specificity to that of Cdc25C to produce a Cdc25C and 14-3-3 protein complex (Kino et al., 2005). Thus, Cdc25C is prevented from entering the nucleus and the cell cycle is arrested. This relationship between Vpr and 14-3-3 proteins is responsible for the first of two major roles of Vpr in leading to SMA, blocking the effects of insulin on forkhead transcription factors (FOXO).

Vpr Blocks Insulin Effects on FOXO

In a normal cell, insulin activates the PI3K/AKT pathway via phosphorylation. This leads to the phosphorylation of the serine and threonine residues of FOXO, which create binding sites for 14-3-3 proteins (Kino et al., 2005). When these 14-3-3 proteins bind to the appropriate sites, FOXO is prevented from passing into the nucleus and transcribing the ubiquitin ligase, Atrogin-1, which is responsible for protein degradation and ultimately SMA (Goldberg et al., 2004).

Therefore, in order to antagonize the effects of insulin on FOXO, Vpr must prevent 14-3-3 proteins from binding to serine and threonine residues of FOXO. By binding to the 14-3-3 proteins, Vpr is competitively inhibiting the crucial role that 14-3-3 proteins have on FOXO. This not only allows FOXO into the nucleus to transcribe Atrogin-1 but also terminates the translocation activity of 14-3-3 proteins which would allow FOXO already in the nucleus to be exported back into the cytoplasm (Kino et al., 2005).

Vpr and Glucocorticoid Hypersensitivity

Vpr directly binds to the glucocorticoid receptor (GR) on a cell membrane, which potentiates the action of the GR on its responsive promoters. In doing so, Vpr acts as a nuclear receptor co-activator along with the host cell's co-activator p300/CBP. This binding and co-activation of Vpr to the GR leaves the latter highly sensitive to the presence of glucocorticoids (Kino et al., 2004).

If the GR is highly sensitive, the cell acts as though a large quantity of glucocorticoids is present. In this case, levels of insulin or the insulin growth factor (IGF-1) decrease. The lack of IGF-1 leads to the dephosphorylation and deactivation of the PI3K/AKT pathways. Subsequently, FOXO is not phosphorylated and therefore allowed into the nucleus where it transcribes the ub-ligase Atrogin-1 (Goldberg et al., 2004). Therefore, by creating a level of hypersensitivity for the GR, Vpr triggers a mechanism leading to SMA.

Indirect Pathways from HIV to SMA

The high levels of cytokines, specifically tumor necrosis factor-_ (TNF- _), which is known to activate the nuclear factor NF_B, are characteristic of HIV. Activation of this pathway stimulates cachexia in the same way as cancer-associated cachexia which leads to protein degradation.

Another role of TNF-_ is that of development and maintenance of inflammation during infection. In the gastrointestinal tract (GI tract), TNF-_ causes local mucosal inflammation which leads to extreme oxidative stress within the body. Oxidative stress induces SMA through many different mechanisms.

Lastly, HIV is correlated with insulin resistance. Insulin resistance is one of the characteristics of diabetes. Therefore HIV can lead to diabetes which leads to SMA.

Cancer Cachexia and SMA

Muscle degradation occurs in cancer cachexia through the IKK/NF_B pathway and the ubiquitin ligase MURF1. This pathway promotes tumor formation. Therefore, the muscle loss in cancer cachexia is not because of the associated anorexia but is in response to the tumor (Diffee et al., 2002).

The transcription factor NF_B is held in the cytoplasm when it is bound to I_B_. In response to an outside stress, such as a proinflammatory cytokine, I_B phosphorylates the serine residues S32 and S36 on I_B_, which causes it to degrade (Cai et al., 2004). NF_B is no longer held in the cytoplasm and it translocates to the nucleus. As a transcription factor, it regulates MURF1 protein production and MyoD mRNA. MURF1 levels increase and MyoD levels decrease. NF_B also induces transcription of its own inhibitor, I_B_ (Ladner et al., 2003).

In a study done by Cai et al. (2004), mice were engineered to have constitutively active I_B (MIKK mice) or to have the I_B_ phosphorylation sites blocked (MISR mice). To simulate conditions of cancer cachexia, they induced tumor growth outside of the muscle. The NF_B activity increased by 6-fold in wild

type mice whereas MISR mice had no significant change. The NF_B was unable to translocate to the nucleus, because it was still bound to the I_B_. In MIKK mice, muscle mass and cross-sectional area of muscle fibers were significantly lowered when compared to wild type mice. In these mice, the percentage of amino acids and their metabolites was increased by 45% which indicates increased protein catabolism. The constitutively active NF_B pathway in these mice caused SMA.

During their study, atrogin-1 was neither stimulated nor inhibited, suggesting that NF_B is a distinct yet parallel pathway.

How do cytokine levels affect SMA?

Tumor Necrosis Factor-_ (TNF-_) is a proinflammatory cytokine and activates the NF_B pathway in a biphasic manner (Ladner et al., 2003). The first transient phase is short, about one hour, while the second phase lasts for 24-36 hours. The second phase is only activated if the TNF levels are high throughout the entire period of time. This occurs during cancer cachexia which explains the constant activation of the NF_B pathway. It was mentioned before that NF_B transcribes its own inhibitor, I_B_. After its transcription, I_B_ is transported to the nucleus and binds to NF_B, which causes the complex to move back into the cytoplasm. The second phase is the more potent of the two and the major cause of muscle degradation.

It is unknown how TNF activates NF_B, although it may go through the aforementioned IKK pathway. It is known that TNF causes the increase in the level of MURF1 and decreases the level of MyoD mRNA, causing skeletal muscle atrophy. Ladner et al. (2003) study found that TNF alone was unable to cause full skeletal muscle atrophy. However, when it was coupled with the cytokine INF_, the results were much more drastic. This suggests that multiple cytokines are necessary for degradation.

What is the substrate of MURF1?

The actual substrate of MURF1 in skeletal muscle is unknown. In a study conducted by Kedar et al. (2004), MURF1 was found to bind to troponin I in cardiac muscle. MURF1 decreases the level of troponin I by inducing its ubiquitination and proteasome-dependent degradation. Troponin I is present in skeletal muscle, but it is unknown whether or not MURF1 binds to troponin I outside of the cardiac muscle.

Why is no new muscle forming?

The NF_B pathway also prevents skeletal muscle differentiation and therefore synthesis of new muscle (Diffee et al., 2002). NF_B prevents skeletal muscle differentiation by inhibiting the production of MyoD mRNA. MyoD helps control the fusion of myoblasts into multinucleated myotubes. Without MyoD, an injured muscle cannot repair itself after damage, but a lack of MyoD has not been shown to inhibit development.

When TNF is present in undifferentiated myocyctes the level of MyoD mRNA is drastically reduced (Diffee et al., 2002). Other differentiation proteins were unaffected. The same results occured for differentiated myocytes and mouse muscle in vivo. TNF and the NF_B pathway reduce the level of MyoD mRNA and therefore leave the muscle unable to repair itself.

NF_B not only causes muscle degradation but prevents synthesis of new muscle, making cancer cachexia a deadly disease. In fact, one-third of cancer related deaths occur because of cachexia, not tumor burden (Guttridge et al., 2000). Direct inhibition of the NF_B pathway may help reduce the muscle loss associated with cancer cachexia.

Oxidative Stress and SMA

Oxidative stress regulates atrophy pathways during periods of prolonged disuse. We will use disuse as model to understand what occurs when symptoms of HIV/AIDS introduce oxidative stress.

Experimental Models

Two models are used to study the mechanism of atrophy in humans. Rat hindlimb suspension models demonstrate immediate decrease in protein synthesis on the onset of unloading, which becomes steady at approximately 48 hours. Slow-twitch fibers (type I) are observed to atrophy more readily than any other muscle type in this form of unloading (Powers et al., 2005). Another disuse model investigates diaphragm wasting by a mechanical ventilator (MV). The MV completely inactivates the diaphragm. This is clinically significant because diaphragm atrophy contributes to difficulty in weaning from the MV. Fast-twitch fibers (type II) are degraded in the diaphragm by MV (Shanely et al., 2002).

Proteolytic Pathways in SMA

There are three proteolytic pathways in the skeletal muscle caused by ROS that can be activated a variety of ways. Some of these pathways work together and some work separately. These pathways involve calcium activated lysosomal proteases (i.e. calpain), endoproteases like caspase-3, and the 20S/26S proteasome system (Powers et al., 2005).

These components are unique features in the three proteolytic pathways. Proteases are known to degrade proteins and, in some cases, lead to apoptosis (Wang et al., 1998). Calpain is in the class of ubiquitous proteases (Koh et al., 2000). Both caspases and calpain belong to a group of cysteine proteases. Cystein proteases are sensitive to the redox condition of a cell. These enzyme proteases have the ability to cleave other proteins. Caspases are pivotal factors in cellular apoptosis (Wang et al., 1998). The 20S/26S proteasome system degrades actomyosin complexes after cleavage by calpain or caspase-3.

Mediation of Intracellular Calcium Levels

There are two known theoretical mechanisms that work together to regulate intracellular calcium levels. The first mechanism involves disuse within skeletal muscles. Muscular inactivity has been shown to increase oxidative stress (Shanely et al., 2002). Reactive oxygen intermediates play a role in the formation of reactive aldehydes (i.e. 4-hydroxy-2,3-trans-non-enal) which decreases activity of plasma membrane calcium ATPase activity (Powers et al., 2005). The inactivity of this enzyme hinders the movement of calcium in and out of the cell. The second mechanism elicits the indefinite presence of calcium within the cell. The sarcoplasmic reticulum is the primary source of calcium for skeletal muscles. In the presence of ROS, antioxidant proteins such as heme oxygenase-1 (HO-1) are upregulated. Calsequestrin, which sequesters calcium to the cell interior, is also

upregulated. Careticulun, however, which inactivates calcium in high capacity and low affinity, is not upregulated. ROS actually reduces the function of careticulun as well (Hunter et al., 2001). In short, these two mechanisms translate to a high concentration of intracellular calcium. This investigation is significant because the activity of the proteolytic pathways mentioned above depend on a high concentration of calcium.

Calpain-Mediated Release of Myofilaments

The first proteolytic pathway activates a lysosomal or cysteine protease, calpain, by the increase of intracellular calcium (Hunter et al., 2001). Calpain then cleaves structural proteins that anchor actomyosin complexes within the sarcomere of the myofibril. These anchor proteins are known as talin and vinculin. The cleavage of talin and vinculin release actomyosin complexes. The third proteolytic pathway mentioned, 20S/26S proteasome, is now apt to degrade the actomyosin complex leading to the onset of skeletal muscle atrophy. The 20S/26S proteasome cannot degrade proteins in the sarcomere unless they are ubiquinated (covalently marked) for degradation by calpain (Powers et al., 2005).

Calpain Inhibitor

It is essential to note that calpain can be inhibited by another molecule. Calpastatin is a regulatory protein that binds to calpain in the presence of calcium to inhibit its function (Koh et al., 2000). Overexpression of calpastatin in a transgene mouse has been shown to reduce muscle atrophy by 30% (Tidball et al., 2002). Another note shows that calpastatin breakdown precedes apoptosis (Wang et al., 1998). This is significant because it shows that a high concentration of calpastatin indicates myofibril viability and the lack of it precedes cell death.

Caspase-3 and SMA

The second proteolytic pathway mentioned involves the endoprotease caspase-3. Caspase-3 can be activated in two ways. Both ways stem from the presence of ROS. In the first way, caspase-3 can be activated by increased calpain activity. Caspase-3 has the same cleavage properties of calpain and has been shown to cleave to the same sites of talin and vinculin (Wang et al., 1998). The second way that caspase-3 can be activated is by the release of cytochrome c from the mitochondria. Cytochrome c is an essential heme protein in the electron transport chain (Powers et al., 2005). Superoxide anions (ROS) are a by-product of mitochondrial electron transport (Reid et al., 2001).

Diabetes and SMA

As previously stated, scientists have noticed a correlation between symptoms in diabetes and SMA. The main principle that scientists are currently trying to resolve is what the correlation between symptoms of diabetes, such as low available insulin levels, and the increased activation of protein degradation.

What activates the Ubiquitin-proteasome pathway?

In muscle cells of rats, it has been documented that insulin deprivation increases protein degradation and that higher insulin levels suppress this degradation (Mitch et al., 1999). Studies suggest that insulin levels or insulin resistance activates some mechanism for protein degradation (Mitch et al., 1999).

Mitch et al. (1999) hypothesized that insulin levels are a signal for activation of an ubiquitin-proteasome pathway. This was tested against acidosis and gluccocorticoid signaling in rats with induced diabetes. First, normal rats were given oral NaCl or $NaHCO_3$ to develop acute acidosis. The rate of protein degradation was much higher than in the control and that when inhibitors of lysosomal or Ca^{2+} dependent proteolysis the rate did not differ. This suggests that the ubiquitin pathway is activated for protein degradation (Mitch et al., 1999).

To ensure that the ubiquitin-proteosome pathway was indeed activated, diabetic rats were fed NaCl or $NaHCO_3$ and the rate of protein degradation was compared to non-diabetic rats with acidosis. The indicated showed that rates of protein degradation between the two groups did not differ significantly.

The same experiment was repeated using gluccocorticoids and similar results were found. Then the diabetic rats were tested after treatment with insulin for three days. The rate of protein degradation was lower in insulin treated rats than in non-insulin treated diabetic rats (Mitch et al., 1999).

These results indicate that the lower insulin level possibly signal the activation of the proteasome pathway. Levels of ubiquitin mRNA were also measured in the previous study and found to be higher (Mitch et al., 1999). This further supports the idea that low insulin levels activate a ubiquitin-proteasome pathway.

Results of Pathway Activation

Goldberg et al. (1999) measured the amount of Ub–conjugation in diabetic rats against that in non-diabetic rats because ubiquitins tag proteins for degradation. The results showed that the amount of Ub-conjugation by E3-_ Ub-ligase in diabetic rats was greater than in non-diabetic rats.

It was also found that the amount of degradation was sensitive to proteasome inhibitors. For that reason, it was concluded that both the amount of conjugation and the rate at which conjugation occurred increased. It also suggested that there must be a third factor that leads to SMA in diabetes.

Role of Proteasomes

With low insulin levels, the amount of gluccocorticoids in the blood, i.e. gluclose, increases. This increase in the gluccocorticoids and the activation of ubiquitination then catalyzes

the formation of the 26S proteasome complex (Attaix et al., 2004). The 26S proteasome is known to degrade proteins that have been Ub-conjugated. It has been observed that during protein degradation in cell cultures, there are high levels of the 26S proteasome (Attaix et al., 2004). The question, then, is what is missing in between.

To test this, rats were treated with DX, a synthetic gluccocorticoid, and their muscles were extracted for examination. Because the 26S proteasome is made from the 19S and 20S components, and the 26S proteasome is known to be highly expressed during atrophy, the levels mRNA for the two components were examined with high levels of gluccocorticoids. Results showed that the mRNA levels for the two components rose as well as the enzymes that are known to work in conjunction with the proteasome.

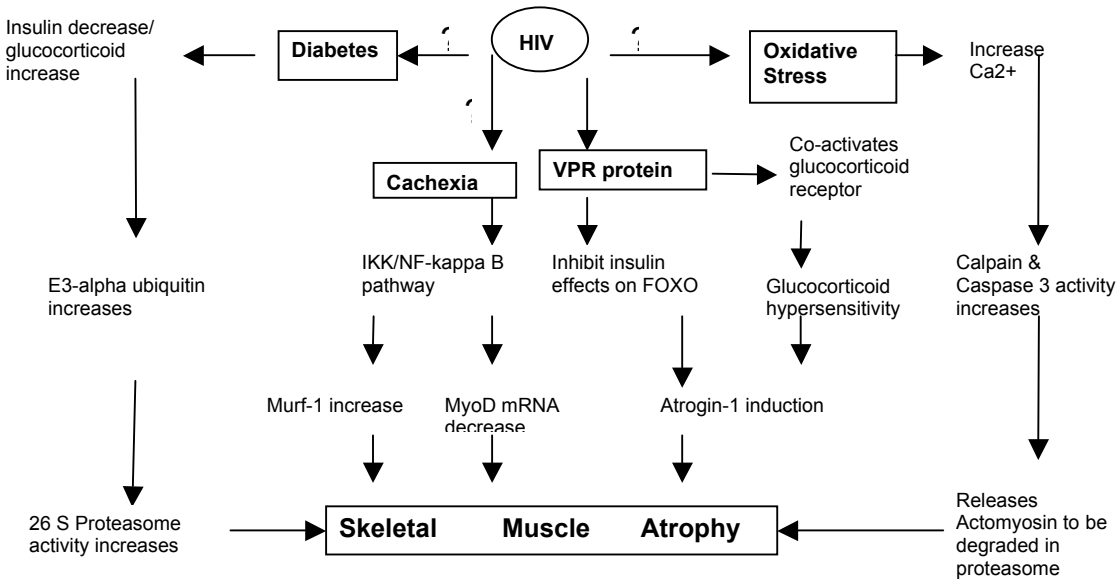

Figure 2. Identifies pathways leading from HIV to Vpr production, Diabetes, Cachexia, and Oxidative Stress.
These effects of HIV then lead through different mechanisms to SMA. Murf-1 Ub-ligase is induced by the NF_B pathway, which is active in both oxidative stress and cachexia. Both mechanisms of Vpr produce atrogin-1, and diabetes induces the E3-_ Ub-ligase. These ubiquitin ligases lead to protein degradation and SMA.

This indicates that diabetes is linked to SMA through the activation of the ubiquitin-proteasome pathway. First, low levels of insulin cause an increase in the amount of gluccocorticoids (Mitch et al., 1999). From the gluccocorticoids, there is an increase in ubiquitination of muscle proteins (Goldberg et al., 1999). Because of the increased activity in the ubiquitin pathway, there is increased transcription for the components of the 26S proteasome. Increased proteasome formation in conjunction with increased Ub-conjugation leads to a rapid protein degradation or SMA (Attaix et al., 2004).

Therapies

SMA is a symptom of a variety of diseases and disorders. Treatments are dependent on the pathology of the disease.

Therapies for SMA in HIV are diverse; they include appetite stimulates, anabolic andrgoenous steroids, resistance training, and some growth hormones and cytokine suppressors. These therapies are designed to treat the wasting syndrome and do not treat HIV.

In cachexia, sodium salicylate is being tested as a pharmacological inhibitor for the NFkB pathway (Cai et al., 2004). This method, however, has not yet been approved.

There are three main therapies for treating SMA in oxidative stress. Nitric oxide has been shown to inhibit calpain cleavage of talin and vinculin. Vitamin E can reduce immobilization-induced muscle atrophy by approximately 20% (Powers et al., 2005). Finally, a therapy mentioned before, expression of calpastatin transgene can cause reduction of muscle atrophy by 30% (Tidball et al., 2002).

The main treatments for diabetes are well studied and revolve around insulin regulation, and insulin regulation would lead to regulation of SMA in patients.

Conclusion

HIV, cachexia, oxidative stress, and diabetes each express direct mechanisms leading to SMA (Figure 2). Each of these diseases or disorders involves the activity of ubiqutin ligases and proteosome complexes. These activities are supported by many studies to be the characteristic mechanism to protein degradation. The direct mechanisms leading to SMA from HIV, cachexia, oxidative stress, and diabetes have been well studied within the past few years. However, the connections between HIV to cachexia, oxidative stress, and diabetes should be studied more in depth. With diseases such as sarcokaposi, an AIDS-specific cancer, a direct pathway from HIV to cancer may be evident. The relationship between diabetes and oxidative stress need to be studied to find specific links leading from HIV. If direct pathways can be identified, HIV could be used as a model to investigate many more mechanisms leading to disease and ultimately to SMA.

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