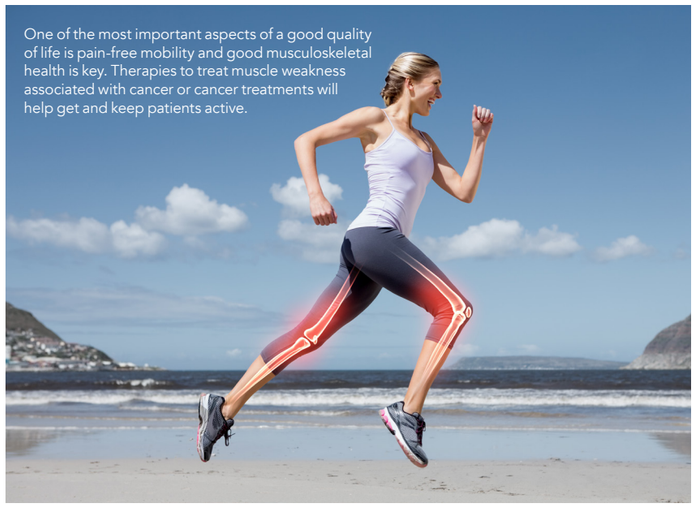
Muscle and bone:

new treatments for cancer-induced muscle weakness

Bone and muscle are two closely connected tissues yet the mechanisms linking them at the cell and molecular level are not well understood. Dr David Waning is an Associate Professor in the Department of Cellular and Molecular Physiology at the Penn State College of Medicine who studies the biochemical mechanisms that cause problems in these tissues during disease and ageing. His lab is developing new treatment approaches that aim to improve musculoskeletal health for cancer, ageing, and chemotherapy-induced conditions.

Muscle weakness is a major clinical problem for patients with advanced cancer. In addition, chemotherapy itself can cause muscle weakness and this can persist for months or years after treatment, causing problems such as fatigue and falls which can lead to fractures and increased mortality. Muscle weakness can occur in the absence of weight loss or in the context of significant muscle wasting. Cancer patients with muscle depletion are also more prone to severe drug-associated toxicity and show a poorer prognosis overall. Currently there is no effective treatment for muscle weakness and the condition is not widely recognised by health care providers.

Tumours which metastasise or spread to the bone are common with many advanced cancers and affect 450,000 patients in the USA each year. Because bone and muscle are so closely interconnected and because muscle weakness is present in patients with these cancers, Dr David Waning and his colleagues began studying muscle weakness in animal models of bone metastases.



One of the most important aspects of a good quality of life is pain-free mobility and good musculoskeletal health is key. Therapies to treat muscle weakness associated with cancer or cancer treatments will help get and keep patients active.

Dr Waning, working with Drs Theresa Guise and Khalid Mohammad at the Indiana University School of Medicine, have unravelled one of the molecular mechanisms linking muscle weakness and cancer-induced bone resorption. Dr Waning is now focusing on the link between chemotherapy and muscle weakness.

THE RELATIONSHIP BETWEEN BONE AND MUSCLE

Historically much more attention has been paid to the physical interactions between bone and muscle than to the biochemical links between them. The bone is shaped by muscles, but bone also conversely affects the shape and size of muscles by providing an attachment site for locomotion. Bone and muscle are tightly connected during growth, and during ageing bone and muscle breakdown also occurs together. If one of the tissues is affected, the other is affected as well. More recently, a deeper appreciation for the molecular connections between bone and muscle has emerged.

The bone is a storehouse for minerals and various types of proteins. In healthy adults, bone is constantly broken down or built up in order to adjust for physical demands on the body or to repair tiny fractures that occur as a part of normal activity. Cells in the bone called osteoblasts, which make new bone, and osteoclasts, which break down and resorb bone, strike a critical balance to maintain homeostasis. When this balance is lost, such as during tumour growth in bone, abnormal new bone can be formed or overall bone loss can occur. When the mineralised bone matrix is broken down, proteins stored there can be released and passed onto the skeletal muscle and can have significant effects on muscle function.

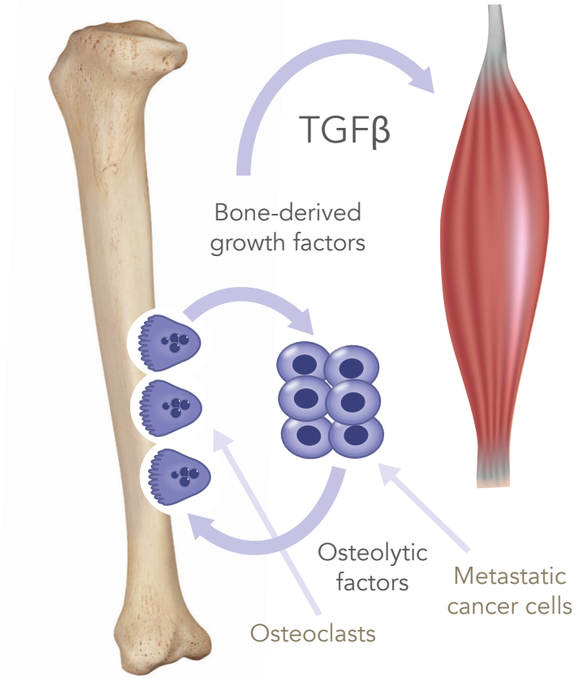
EXCESS BONE RESORPTION IMPAIRS MUSCLE FUNCTION

When certain cancers spread to the bone, tumour cells release proteins that aid their growth in bone and lead to the formation of more osteoclasts. The process of breakdown and resorption of bone by osteoclasts releases other proteins called growth factors from the mineralised bone matrix. One such protein growth factor is TGFβ, which stimulates tumour growth in metastasised breast cancer and stimulates further bone breakdown by osteoclasts. This results in a vicious cycle that leads to bone pain, fractures, excess calcium in the blood, pinched nerves and muscle weakness.

In research published in the journal *Nature Medicine* in 2015, Drs Waning and Mohammad, working with Dr Guise, looked at mouse models of human breast, lung and prostate cancers that had metastasised to bone and multiple myeloma in bone and found that the animals had impaired muscle function. This was not due to the presence of tumour cells in the muscle. The researchers then investigated whether muscle weakness caused by tumours that spread to the bone was due to a deficiency in muscle contraction or solely due to loss of muscle mass.

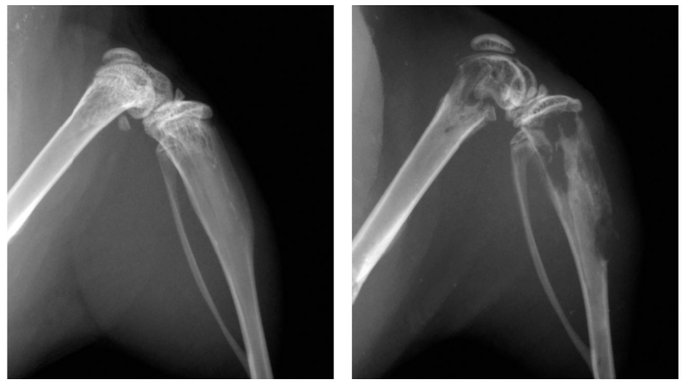
They found that TGFβ, when released from bone, caused levels of a protein called Nox4 to increase. Nox4 is a protein that creates reactive oxygen species (ROS), chemically reactive molecules that contain oxygen. ROS causes damage to the cell and oxidises proteins in the skeletal muscle. One of the targets of Nox4-induced oxidative stress is a protein critical for muscle contraction called RyR1, which functions as a channel for calcium. Collaborating with a team led by Dr Andrew Marks, at the Columbia University College of Physicians and Surgeons, they found that when RyR1 channels were oxidised they leaked calcium, resulting in these channels working incorrectly. As a result, muscle contraction, which depends on calcium, was not able to proceed properly.

The researchers also showed that TGFβ was active in skeletal muscle of people with breast cancer and lung cancer that had metastasised to bone, and that in this

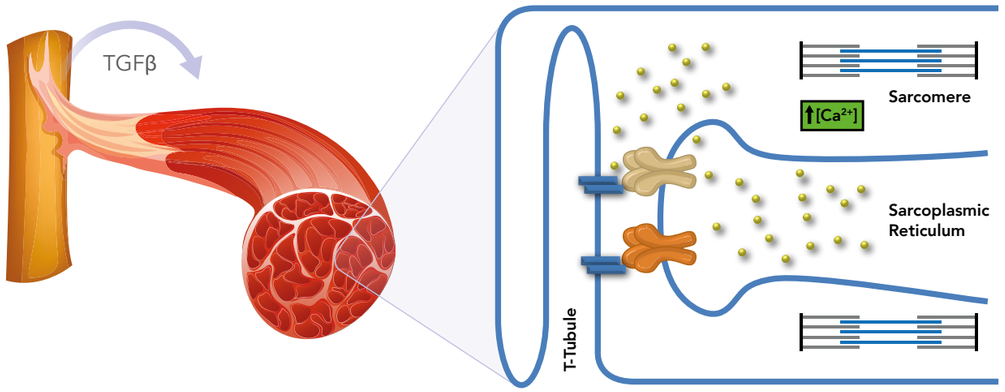


Tumour cells that metastasise to the bone can release osteolytic factors that stimulate the osteoclasts, bone resorbing cells, to increase bone turnover. TGFβ, a cytokine released from the mineralised bone matrix by the activity of osteoclasts, drives a feed-forward vicious cycle of tumour growth in bone and leads to systemic skeletal muscle weakness.

The researchers' data has shown that factors released from the bone matrix cause oxidative stress in muscle and that this is able to cause muscle weakness.



Radiographs of the distal femur and proximal tibia of a healthy mouse (left) and a mouse with osteolytic bone metastases (right). Notice the bone destruction (dark areas) in the leg of a mouse with tumour cells growing in the bone. For bone metastases, 100,000 MDA-MB-231 breast cancer cells are injected into the left ventricle of mice and radiographs are taken after approximately four weeks.



Model of calcium leak in skeletal muscle that causes weakness. Excess TGFβ leads to RyR1 channel oxidation. Oxidised RyR1 channels leak calcium from the sarcoplasmic reticulum, the calcium storehouse in muscle cells, that leads to skeletal muscle weakness due to high cytosolic calcium concentration.

muscle the RyR1 calcium channel is also likely leaking calcium.

When the researchers prevented calcium leakage using a small molecule known as Rycal S107, they saw improved muscle function in mice with bone metastases. This phenomenon was also observed when they blocked TGFβ from signalling in muscle by blocking bone resorption which prevented the release of TGFβ from the bone. The researchers also used various other inhibitors of TGFβ which prevented TGFβ signalling in muscle and the increase in Nox4 protein. The researchers also prevented muscle weakness when they blocked Nox4 activity directly using a small molecule inhibitor.

A similar set of experiments was also done using mouse breast cancer cells in mice. This was done to see if the immune system of mice could be playing a role in muscle weakness, loss of muscle size, or growth of tumour cells in the bone. The work with human cancer cells required injection of the human cells into an immunodeficient mouse often referred to as nude mice since they lack fur. The researchers found the same results using mouse tumour cells as when they used human tumour cells in an immunodeficient mouse, suggesting that the immune system is not playing a large role in cancer-associated muscle weakness.

The researchers concluded that the release of too much TGFβ from bone contributes to muscle weakness by disrupting the normal calcium cycle that is needed to produce muscle contraction. This study demonstrated that the proteins TGFβ, Nox4 and RyR1 are all potential drug targets for cancer-associated muscle weakness.

CHEMOTHERAPY CAUSES MUSCLE WEAKNESS AND BONE LOSS

Cachexia is a condition characterised by weight loss, depletion of fat and muscle, increased fatigue, reduced quality of life and high mortality. In previous studies cachexia has been associated with chemotherapy. However, the molecular mechanisms of this association were not clear. Dr Waning and his team are now looking into the role that chemotherapy plays in the development of muscle weakness and how chemotherapy may be altering energy usage that can affect how muscle is working. They are also looking at changes that chemotherapy causes in bone and how the overall musculoskeletal system is affected by chemotherapy.

With funding from the US National Institutes of Health, through a special review panel for provocative questions in cancer research at the National Cancer Institute, Dr Waning has been working to understand changes in muscle function and cachexia due to chemotherapy. In these studies, the researchers give chemotherapy commonly used for breast cancer to healthy mice to study their muscle and bone. Their data supports the notion that chemotherapy causes oxidative stress in muscle and that this is able to cause muscle weakness and cachexia. They have also found changes in bone related to chemotherapy treatment.

These studies, along with what has been learned about cancer-associated muscle weakness, will provide data to further develop treatments for cancers that use chemotherapy that induces musculoskeletal changes, and these studies have the potential to improve the quality of life and survival of cancer patients.

FUTURE RESEARCH

The goal of Dr Waning's research is to come up with a treatment for muscle weakness, which could help not only those afflicted with cancer who develop this condition, but those with osteoporosis or ageing-related muscle weakness as well. His lab is also actively studying bone-muscle crosstalk in mouse models of cancer and in mice treated with chemotherapy. These studies will give us a better understanding of changes in the musculoskeletal system that could one day be targeted so that people can maintain an active lifestyle that today is not possible.

These studies have the potential to improve the quality of life and survival of cancer patients.

Behind the Research

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Research Objectives

Dr Waning's work focuses on the study of cancer and cancer therapy-associated skeletal muscle weakness and cachexia.

Detail

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Bio

David L. Waning received his PhD from Northwestern University and after several years in the pharmaceutical industry, completed postdoctoral training at the Indiana University School of Medicine. He is currently an Associate Professor in the Department of Cellular & Molecular Physiology at the Penn State College of Medicine.

Funding

NIH

Collaborators

- Dr Khalid Mohammad, MD PhD, Professor, Indiana University School of Medicine
- Dr Theresa Guise, MD, Professor, Indiana University School of Medicine

PennState College of Medicine

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Personal Response

What drove you to study cancer-induced muscle weakness?

" I was actually first interested in studying the communication between tumour cells that had invaded the bone and the local bone microenvironment. In the course of those first experiments, I noticed that mice with breast cancer bone metastases lost a tremendous amount of weight and did not move around as much. To my surprise nobody had ever studied muscle function in these mice. With Dr Theresa Guise, I began studies of muscle contraction in these mice and we found significant weakness that could not be explained by the loss of muscle mass. "