Chapter 3
Research Progress of the Osteogenic Activity of the Active Peptides from *Caulerpa Lentillifera*

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Abstract *Caulerpa lentillifera* is a potential cash crop, the current promotion and cultivation in China is in the development stage. Take *Caulerpa lentillifera* as a raw material to extract the active peptides. Then, investigate the effects of active peptides on the proliferation and differentiation of osteoblasts. Simultaneously, the metabolomics strategies was used to find the different metabolites and metabolic pathways, combining with other molecular biology methods and metabolomics results, to discuss the molecular mechanism of active peptide extracts. This filed of research is to provide scientific basis for the exploitation of marine resources and the development of new anti-osteoporosis drugs.

Keywords Progress · Osteogenic activity · Peptide · *Caulerpa Lentillifera*

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3.1 Introduction

Osteoporosis is a progressive degenerative disease that usually occurs with age. Its pathogenesis is very complex, from the cellular level, osteoblasts are the most important material basis for osteogenesis and bone formation, and its proliferation and differentiation defects are one of the root causes of osteoporosis. At present, many bioactive food factors as well as natural products have been shown to promote the growth of osteoblasts. Among them, some small molecule polypeptides have been shown to have the ability to promote osteoblast proliferation and differentiation. In vitro experiments have also shown that small molecule collagen polypeptides can stimulate the proliferation and differentiation of osteoblasts, and then regulate the expression of osteoblasts related to bone formation, ultimately affecting the growth process of osteoblasts.

Caulerpa lentillifera is beginning to be popularize planted in China, but it has not yet been optimized utilization. Hence, its added-value of products is relatively low. The application and development of *Caulerpa lentillifera* as a cash crop, will provide a scientific basis for expanding the development of marine active peptide resources and new anti-osteoporosis drugs. The extraction of active peptides from *Caulerpa lentillifera*, as well as the research on the activity of osteogenesis are rarely reported in the literature at home and abroad.

3.2 Progress of the Treatment of Osteoporosis

Osteoporosis is a progressive degenerative bone disease that usually occurs with age (Payal et al. 2017). It is characterized by decreased bone mineral density and quality, destruction of bone microstructures, and increased bone fragility caused by a variety of factors that predispose to fractures (Sozen et al. 2017). Osteocellular cells are the smallest units that make up bones, and osteoblasts are precursors of bone cells, and their reproduction and differentiation are of great significance to the growth of bones (Fu et al. 2019). The pathogenesis of osteoporosis is very complex, from the cellular level, osteoblasts are the most important material basis for osteogenesis and bone formation, and their proliferation and differentiation defects are one of the root causes of osteoporosis. Therefore, the key to treating osteoporosis lies in how to promote the proliferation and differentiation of osteoblasts. At present, traditional estrogen replacement therapy can promote the proliferation and differentiation of osteoblasts, which has a good early effect on osteoporosis. However, its long-term safety has been widely questioned. In the search for alternatives to estrogen, many bioactive food factors (calcium, vitamin D3, collagen, etc. (Eaton-Evans 1994)) as well as natural products (icariin (Huang et al. 2018) and kaempferol (Kim et al. 2016)) have been shown to promote the growth of osteoblasts. Type I collagen, which is widely distributed in connective tissues such as ligaments and cartilage, plays an important role in the reproduction and differentiation of osteoblasts. However, collagen

has a special triple helix structure (Villa et al. 2016) and a large molecular weight (Sneha et al. 2016), which is often detrimental to the digestion and absorption of organisms. Conversely, some small molecule polypeptides (such as casein derivative active peptides (Reddi et al. 2018) and whey protein-derived peptides (Pandey et al. 2018)) have been shown to have the ability to promote osteoblast proliferation and differentiation. In vitro experiments have also shown that small molecule collagen polypeptides can stimulate the proliferation and differentiation of osteoblasts, and regulate the expression of osteoblasts-related genes related to bone formation, ultimately affecting the growth process of osteoblasts (Daneault et al. 2015). Therefore, peptides can promote the proliferation and differentiation of osteoblasts more than collagen.

A polypeptide is an active substance that is between amino acids and proteins, with amino acid numbers of 10–100. In recent years, its physiological functions have received more and more attention. The polypeptide, its structure is simpler than protein, it is easy to absorb with hypoallergenic and other advantages, it is widely used in clinical practice and can provide nutrition for the organism. At present, there are two main types of peptide products: one is a polypeptide drug or a polypeptide reagent, and the other is a health food containing a functional factor active polypeptide or an ordinary food containing an active peptide. With the rapid development of modern science, technology and equipment, the production cost of active peptides has dropped significantly, and active peptide foods and drugs have entered the golden stage. Studies have shown that the active peptide obtained by hydrolysis has a certain effect on the proliferation and differentiation of osteoblasts, and also has a certain prevention and control effect on improving osteoporosis.

3.3 Research Progress of *Caulerpa Lentillifera*

Caulerpa lentillifera is an edible seaweed grown in tropical waters and is named sea grape because of its appearance like a bunch of crystal clear green grapes (Nguyen et al. 2011). The texture of sea grapes is quite similar to salmon roe, but it is fresh and fragrant, and there is no fishy smell of fish eggs, so it is known as the green caviar in the plant, which is the perfect match of sushi, and it is very popular on the Japanese table. Sea grapes belong to the fern family of green algae, mainly distributed in Okinawa, Indonesia, Vietnam and other Asian countries (Bhesh et al. 2015). It is rich in a variety of nutrients needed by the human body, including amino acids, unsaturated fatty acids, minerals, and is also rich in macro or trace elements such as P, Ca, Mg, Cu, and Se (Paul et al. 2014). Studies have shown that 80% of *Caulerpa lentillifera* are composed of seaweed polysaccharides, collagen and dietary fiber. Among them, the protein content is significantly higher than that of terrestrial vegetables (about 10.4–14.7%), and the amino acids are relatively complete. It has a higher content of polyunsaturated fatty acids (about 16.7%) (Matanjun et al. 2009) and dietary fiber (about 8%), while its total fat content is lower (1.6–3.7%) (Tong et al. 2022), making it a class of marine cash crops with certain health functions.

At present, there are few domestic studies on *Caulerpa lentillifera.* There are some research on the extracts of *Caulerpa lentillifera.* Sharma, et al. found that the extracts of *Caulerpa lentillifera* could improve insulin resistance and regulate glucose metabolism in mice through the PI3K/AKT signaling pathway, suggesting that *Caulerpa lentillifera* extracts might be used for diabetes prevention and control (Bhesh et al. 2015). Other studies have shown that the extracts of *Caulerpa lentillifera* were effective in inhibiting cell death, significantly improved insulin secretion and expression of fat cell glucose transporters as well as the and glucose uptake (Bhesh and Dong 2014). Japanese scholar Maeda, et al. used water as an extractant to extract *Caulerpa lentillifera* polysaccharides, and found that the extracted polysaccharides could increase the expression of genes encoding cytokines through the p38 MAPK and NF-κB signaling pathways, thereby enhancing the phagocytic activity of macrophages (Maeda et al. 2012). The NF-κB mentioned in the above-mentioned study plays an indispensable role in normal physiological processes such as the immune system, skeletal system, and epithelial tissue by regulating cell reproduction, differentiation, and proliferation (Wullaert et al. 2011). NF-κB plays an important regulatory role in the skeletal system, and its important influence on the differentiation and maturation of osteoblasts and osteoclasts and their functional activities (Boyce et al. 2010), making the NF-κB signaling pathway a new research target for the treatment of diseases with abnormal bone mass growth or reduction (Chang et al. 2011).

The relatively high protein content and complete amino acid range of *Caulerpa lentillifera* have been preliminarily confirmed to have important biological activities. Do the peptide actives contained in *Caulerpa lentillifera* have high osteogenesis activity? Is there a therapeutic effect on osteoporosis? Unfortunately, there are still few reports at home and abroad on the active peptide of *Caulerpa lentillifera*, and the research on the extraction process of its active peptide is basically in the blank, and there are no reports on the osteogenesis activity of the *Caulerpa lentillifera* active peptide. Therefore, the systematic study of the extraction, isolation, purification of the active peptides of *Caulerpa lentillifera* and their biological effects on osteoblasts has become an urgent scientific and technological topic to be solved.

3.4 Metabolomics Technology Combined with Multi-technology Used to Evaluate the Cellular Biological Effect of Active Peptides

There are many ways to evaluate the cellular biological effects of biologically active substances. As a high-throughput research tool, metabolomics can be used to detect endogenous small molecules in the products of biochemical reactions, to illustrate the connections between different pathways operating in a living cell (Thomas et al. 2011). Therefore, metabolomics is often used to reflect and evaluate differences in biochemical indicators between healthy or pathological organisms.

In addition, cytometomics can provide a direction for drug development or intervention (Wang et al. 2012). Cell metabolomics can be used to qualitatively and quantitatively describe the final response of biological systems to genetic factors or changes in the external environment by detecting final metabolites regulated within the cell (Miroslava et al. 2010). At present, the metabolomics of cells has been widely used in toxicology, efficacy evaluation, cell culture monitoring, new drug research, biopharmaceutical production, food omics and other fields. In addition, in vitro cell metabolomics has many advantages such as easy operation, high reproducibility, low cost, and easy tracking of results. In recent years, advances in the study of cellular metabolomics for evaluating the biological effects of active substances have been widely recognized. Omics technology was used to detect the metabolism of osteoblasts co-cultured with active peptides of *Caulerpa lentillifera*, and combined with molecular biology techniques, it was analyzed from the aspects of gene and protein expression regulation to further verify the metabolomics results. At the same time, combined with the proliferation, differentiation and other phenotypic information of osteoblasts, multi-technology combination to evaluate the osteogenetic activity of *Caulerpa lentillifera* active peptides. It will be useful to reveal the molecular mechanism of the biological effect of the active peptide on bone cells of the *Caulerpa lentillifera.*

3.5 The Extraction of *Caulerpa Lentillifera* Active Peptides Can Promote the Development of Osteoporosis Drugs

With the increasingly serious phenomenon of population aging in modern society, osteoporosis and its complications have become one of the main diseases that lead to the decline and death of people's quality of life, and are more and more highly valued by people. At present, the treatment of osteoporosis is still an urgent problem to be solved in the clinic. The basic pathogenesis of osteoporosis is that the coupling of bone formation and bone resorption in the process of bone metabolism is unbalanced, resulting in an imbalance in the metabolism of calcium and phosphorus in the body, a decrease in osteogenesis capacity and an increase in osteoclast activity, an acceleration of bone resorption, and a gradual decrease in bone density. Therefore, it is of great significance to promote osteoblast proliferation and differentiation, improve osteoblast function and regulate bone reconstruction.

China has a long coastline, coastal islands are dotted with coastlines, and marine resources are abundant. As a common type of seaweed, *Caulerpa lentillifera* is one of the most important component of marine resources, some of its components have special biological activities, there are broad application prospects, and it is imperative to fully carry out the research of *Caulerpa lentillifera.* Due to the high value of *Caulerpa lentillifera* in nutrition, it is widely promoted in Qingdao, Shenzhen and other cities in China. A small number of areas in Fujian province have emerged small-scale of aquaculture of *Caulerpa lentillifera*, but they have not been optimized

utilization, the added-value is relatively low. In particular, the extraction and utilization of active ingredients in *Caulerpa lentillifera* are still rarely studied, the extraction process and application of *Caulerpa lentillifera* active peptides are even rarer in the literature. If the extraction process of the active peptide of *Caulerpa lentillifera* can be optimized most reasonably, and the properties of its active peptide extract can be studied and exploited, it is conducive to sustainable environmental development and will also bring immeasurable economic and social value.

Classify the *Caulerpa lentillifera* active peptide to obtain different levels of active peptide molecules with higher purity. Then, taking osteoblasts as the research object, the proliferation and differentiation of osteoblasts under the culture of *Caulerpa lentillifera* active peptide extracts were analyzed. The influence mechanism of *Caulerpa lentillifera* active peptide on the metabolic pathway of osteoblasts was explored by combining metabolomics strategies, combined with molecular biology techniques, and multiple technologies such as gene and protein expression regulation. Focusing on the main circulation pathways that are closely related to the local regulation of osteoblasts, such as the Wnt signaling pathway, the bone morphogenesis protein signaling pathway (BMP), and the mitogen-activated protein kinase (MAPK) signaling pathway, and using this as the starting point to explore the mechanism of action of *Caulerpa lentillifera* active peptides on osteoblasts, it will provide a scientific basis for the development and utilization of marine algae resources and the development of new anti-osteoporosis drugs.

3.6 Prospection

Caulerpa lentillifera is beginning to promote planted in China, but it has not yet been resource utilized, and the added-value of products is relatively low. The extraction and utilization of its active ingredients will broaden the channels for the application and development of this cash crop. The extraction of active peptides of *Caulerpa lentillifera* has not been reported in the literature at home and abroad.

The evaluation of *Caulerpa lentillifera* active peptide on osteogenetic activity by metabolomics strategies combined with multiple molecular biological methods was not reported. Research on *Caulerpa lentillifera* active peptide will provide a scientific basis for expanding the development of marine peptide resources and the development of new anti-osteoporosis drugs.

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