氏名（本籍）	Sajal AFZAL
学 位 の 種 類	博士（病態機構学）
学 位 記 番 号	博 甲第　9995　号
学位授与年月	令和　3　年　3　月　25　日
学位授与の要件	学位規則　第４条第１項該当（昭和２８年４月１日文部省令第９号）
審 査 組 織	グローバル教育院
学位論文題目	Molecular Mechanisms of the Antistress and Anticancer Activities in Some Natural Compounds （いくつかの天然化合物における抗ストレスおよび抗癌活性の分子メカニズム）

	（職名）	（学位）	（氏名）
主査	筑波大学准教授	理学博士	坂本　和一
副査	筑波大学教授（協働大学院）	博士（生物学）	Renu Wadhwa KAUL
副査	筑波大学教授	博士（農学）	礒田　博子
副査	筑波大学准教授	博士（医学）	許　東洙

論文の要旨
Abstract of thesis

Stress is a physical factor that is perceived by a living cell as undesirable. It may be environmental (exposure to extreme temperatures, radiations, chemicals, or heavy metals), or internal (hormonal fluctuations, temperature variations, or chemicals). It evokes molecular and metabolic changes in living cells leading to adaptation to the new environment. Failure to do so leads to growth arrest or apoptosis of cells. At organismal level, it plays an important role in determining the quality of life and aging parameters. Often, physiological functioning of cells and tissues is afflicted in a way that it leads to pathological conditions including organ dysfunctions. Most commonly stress is connected to either brain-related disorders such as neurodegenerative diseases, or a variety of cancers or even premature aging. These disorders are categorized under stress-related disorders; their prevalence around the world is continuously rising. Physical, social, and mental stresses are some of the largest causes of ill-health. With expanding average lifespan, growing environmental stresses, global warming, machine-like lifestyle, evolving microbes, and the emergence of sophisticated disease patterns, the stress responses are rapidly increasing and unlikely to subtle. Cancer incidence has risen to about 1.7 million new cases and 6 hundred thousand deaths in 2018, annually in the USA alone and to about 18 million new cases and 9.5 million deaths in 2018, annually globally. Neurodegenerative diseases, including Alzheimer’s diseases, Parkinson’s diseases, and Amyotrophic Lateral Sclerosis primarily, absorbing millions of patients worldwide, grow in incidence with age, have a sporadic and familial origin, and increase with exposure to stresses in the early age. Since the signs and symptoms of these disorders are minute and often discrete in the early stages, only their late stages or their complications are diagnosed and left to treat. This poses a major hurdle for clinicians nowadays, thereby warranting interventions to prevent or delay the

onset of these diseases. One of the solutions to these problems could be the preventive measures such as use of nutraceuticals and herbs. Synthetic medicines face severe drawbacks; they are expensive and cause adverse effects. On the other hand, natural molecules offer affordable and easily available therapeutic plans with high tolerability and broad-spectrum actions. Although a variety of nutraceutical components and herbs have been reported for their good effects on health, the knowledge on the specific effects and molecular mechanism of action have not been enough described. The author reports cellular and molecular studies on effect of some natural compounds (Carotenoids- Astaxanthin and Fucoxanthin, and Resveratrol) on cell functions in normal and stressed conditions.

Human normal and cancer cells were used as model system. Various assays including cell proliferation, growth arrest, apoptosis, migration and differentiation were performed using specific reagents and tools. Cells were cultured in normal and stressed conditions during which the viability profiles were first determined. Effect of selected compounds was determined on cell proliferation, migration and differentiation under a variety of conditions. Astaxanthin and fucoxanthin are marine carotenoids found commonly in red algae and seaweed, respectively. They are known for their potent antioxidant mechanisms, thus were expected to offer benefit in stress-related disorders. The author reported extensive analyses of the bioactivity of fucoxanthin and astaxanthin. It was found that fucoxanthin, but not astaxanthin, possess good anti-migratory potential. Human colon cancer cells harboring a mutant isoform of p53 showed delay in their migration when treated with fucoxanthin. Molecular mechanism of action of this phenotype was investigated and it was found that fucoxanthin caused reduction in mortalin protein that is a critical factor for cancer cell migration and cancer cell stemness. Based on the data, it was suggested that fucoxanthin induced reduction in mortalin is sufficient to afflict the cell migration and hence metastasis of cancer cells. Of note, effect of fucoxanthin on cell migration was confirmed in cells irrespective of the wild type or mutant p53 status of cancer cells. These data suggested that fucoxanthin supplementation would be useful for treatment of aggressive and metastasis cancers. Low doses of fucoxanthin were established that did not affect the proliferation and or migration but showed antistress effects. Particularly significant effect was observed on skin derived cells stressed with oxidation and pigmentation stresses. Based on these data, it was suggested that fucoxanthin has QOL-enhancing potential in normal and stressed states of life. In order to further test this concept, stressed brain-derived cells were used.

Using *in vitro* rat glioma cell model, marine carotenoids (fucoxanthin and astaxanthin) were tested for their differentiation inducing potential. Such effect was also anticipated to be useful for treatment of brain cancers that cannot be treated by surgery and chemotherapy due to technical difficulties and blood-brain barrier. Induction of re-differentiation is thought to offer alternative approach. Both astaxanthin and fucoxanthin when supplemented at their non-toxic (IC_{01}) doses chronically (>1 month) to the astrocytic cancer cells in the cell culture system caused the appearance of the cells morphologically similar to the healthy astrocytes. While their abnormal cell proliferation slowed down with time, they were molecularly supported by activation of the genes and proteins matching the physiologically differentiated cells. With both glial and neuronal protein markers upregulated, the marine carotenoids were interpreted to serve as chronic therapy and caused an immature astrocytic type of re-differentiation in the glioma cells.

In similar lines, another natural compound, resveratrol, a stilbenoid commonly found in grapes, was analyzed. It also caused potent antistress activity in the stressed brain cancer cells. Resveratrol is also known for its antioxidant potential. This investigation reports its neurodifferentiation potential in both rat glioma and human neuroblastoma. Non-toxic (IC_{01}) dose of resveratrol protected the cells against several types of stresses and caused their differentiation to astrocytes in short term and mature neurons in long term assays.

Discovery of the natural compounds and their mechanisms of actions with experimental evidence is helpful for the development of better nutraceutical formulas and help in improving the QOL in normal and diseased scenarios. With the marine carotenoids astaxanthin and fucoxanthin from *Haematococcus pluvialis* and *Undaria pinnatifida*, respectively, and resveratrol from *Vitis vinifera*, this thesis reports the latest scientific findings and recommend further exploration for molecular mechanisms.

審査の要旨
Abstract of assessment result

【批評 Review】

The applicant used cell culture as a model system for her research and investigated the molecular mechanisms of the anticancer and antistress activities of the marine carotenoids fucoxanthin and astaxanthin and berry stilbenoid resveratrol. She successfully used a variety of cancer cells. Majority of the applicant's work describes dose dependent effect of fucoxanthin in cellular assay. Whereas high dose of fucoxanthin caused cytotoxicity and apoptosis in cancer cells by mechanism involving activation of tumor suppressor protein p53, the low dose (non-toxic) doses was found to possess anti-migration activity. Molecular mechanism of such activity was described to involve downregulation of mortalin protein. Taken together the molecular data suggested that fucoxanthin could be recruited to treat the cancer cell metastasis in aggravated disease. The applicant used low dose of fucoxanthin in cell culture assays in normal and stressed conditions and showed that low doses of fucoxanthin have the capability to protect cells from some kinds of stress. Most pronounced change was found as reduction in stress-induced melanogenesis. In order to validate the antistress activity of these natural compounds, the applicant recruited the brain-derived cancer cells and undertook extensive analyses of control and treated cells. Molecular studies clearly elucidated the antistress mechanisms of these molecules. Induction of differentiation was noticed in the treated cultures and extensive study validated the differentiation phenotype and underlying molecular changes. Based on these data, possible use of these compounds for treatment of brain cancers that suffer from hurdles for surgery, radio and chemotherapy, was proposed.

The applicant has compared the effect of fucoxanthin, astaxanthin and resveratrol in cell-based antistress assays and found that these compounds have similar activities to some extent but differ in their mechanism of action. This way, the applicant has opened up the novel aspects of using these natural compounds for antistress and anticancer treatments more specifically for brain cancer. Use of an extensive number of *in vitro* assays, dose response measures and elucidation of molecular responses that supported the phenotypes is the highlight of this thesis and clearly shows the potential of the applicant to design, plan and execute experiments in the field of life-sciences. The work has initiated new leads that could be benchmarked for further research.

【最終試験の結果 Result】

The final examination committee conducted a meeting as a final examination on 13th January 2021. The applicant provided an overview of dissertation, addressed questions and comments raised during Q&A session. All of the committee members reached a final decision that the applicant has passed the final examination.

【結論 Conclusion】

Therefore, the final examination committee approved that the applicant is qualified to be awarded Doctor of Philosophy in Disease Mechanism.