Review on Autophagy researches and its potential applications in pharmaceutical, medical and cosmetic industries

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I. Abstract

Autophagy is a crucial catabolic activity of the cell that degrades and recycles cellular waste products for future uses. The laureate of the 2016 Nobel Prize in Physiology or Medicine, Yoshinori Ohsumi, has devoted many years on elucidating its mechanism and his award has brought the attention of many researchers around the globe to investigate on possible effects of sufficient and insufficient autophagy and their causes. This paper reviews existing studies of autophagy and analyze its applications in different fields, examining potential applications in real-life industries. Interviews with experts and review of literature have provided most content including future research pathways. Limitations involve lack of experiment and reliance on literature and personal opinions. Reviewing current journals has revealed the current stance of autophagy research from elucidation of its mechanisms to its application to human as medicine or beauty product. Though much advancement were made from the discovery of autophagy, all mechanisms of autophagy must be clarified in the molecular lever for its application to be possible in pharmaceutical, medical, and cosmetic industries and become available to customers as products.

II. Introduction

This paper aims to review the progress in autophagy researches regarding its applications on pharmaceutical, medical and cosmetic/beauty industries. With Dr. Yoshinori receiving Nobel Prize on his researches that elucidate autophagy mechanisms, only understanding of theoretical background of this newly found degenerative process allows its application in actual industries. Through review of current stance of autophagy researches, this paper identifies and suggests future pathways of autophagy research by gathering scientific achievements and potentials made until 2016.

Due to limitations of this research of not being able to access a lab, this paper focuses on literary documents and academic journals. Based on journals of choice, interviews were conducted regarding their publications, opinions on future of autophagy, and its potential applications in real-life industries. Interviewees include Dr. Ho Jeong Kwon, professor of Yonsei University in College of Life Science and Biotechnology, Dr. Byung-Wan Lee, professor of Yonsei University in College of Medicine, Dr. Seung-Yong Yoon, professor of Ulsan University in College of Medicine, and Dr. Han Woong Lee, professor of Yonsei University in College of Life Science and Biotechnology.

III. Background on Autophagy

a. Definition of Autophagy

Autophagy is a self-degradative process that is important for balancing sources of energy at critical times in development and in response to nutrient stress.[1] It plays a significant role in maintaining

the homeostasis of the body by removing mis-folded or aggregated proteins, which can cause amyloidosis.[2] In addition to aggregated proteins, autophagy also processes damaged organelles including mitochondria, endoplasmic reticulum and peroxisomes, and eliminate intracellular pathogens. On that account, it is considered as a survival mechanism of an organism as it degrades and recycles cellular components as nutrients and energy resources. However, studies show that dysregulation of this process lead to type II cell death, a non-apoptotic cell death.[3] Other than its cleaning function, its role in cellular senescence and cell surface antigen presentation have grasped the interests of many researchers for its potential in curing or alleviating certain diseases like cancer.[4, 5] The main type of autophagy is macroautophagy, autophagy referred to in Yoshinori Ohsumi's Nobel Prize-winning work, involves the degradation of cytoplasmic constituents with the fusion of a vesicle called *autophagosome* and lysosome.

b. Discovery of Autophagy

Today, autophagy is well defined by many researchers through numerous studies and experiments, with its type and proteins included in its pathways. It started from the discovery of the end part of the pathway; the discovery of lysosome. In 1955, Christian de Duve discovered a membrane-bound organelle with low pH and hydrolytic activity in experiments with rat liver lysates, and he named it lysosome.[6] In 1956, Alex Novikoff observed similar organelles under

the electron microscope and called them “dense bodies” in not only rat liver lysates, but in various tissues, introducing the idea that the pathway is not constricted to liver tissues. Just a year later, Sam Clark observed mitochondria inside the “dense bodies” in electron microscopy of the developmental stages of mouse kidneys, and suggested that the vesicle did not derive from outside of the cell via endocytosis.[7] Then, Novikoff in 1959 proved that the “dense bodies” are lysosomes by showing that the former in mouse kidneys contains lysosomal enzymes. Observations of lysosomes containing intracellular components like mitochondria and endoplasmic reticulum was made, and in 1963, Christian de Duve finally coined the term autophagy to name the pathway by combining Greek words ‘auto-’ (meaning self) and ‘-phagy’ (meaning eating) at the Ciba Foundation symposium.[8]

In 1990s, scientists started to extensively investigate on autophagy and related genes. One notable scientist is Yoshinori Ohsumi, laureate of 2016 Nobel Prize in Physiology or Medicine. Through numerous publications, Ohsumi defined key genes for autophagy and elucidated the function of encoded proteins in budding yeast. In early 1990s, Ohsumi used yeast, with its vacuole as a counterpart of lysosome in mammals, and was able to show that autophagy existed in yeast with the observation of accumulation of engulfed cytoplasmic components in the vacuole upon inhibition of vacuolar enzymes. In his consequent studies, he discovered 15 genes that activate autophagy in eukaryotic cells, which he named APG1-15,

in 1993. His findings enabled scientists to further study on the function and mechanism of autophagy in higher eukaryotes, and led to discovery of effects of its inhibition or dysregulation on pathogenesis of several diseases.

c. Types of Autophagy

i. Macroautophagy

This type of autophagy is the most well defined pathway until present. Macroautophagy functions to eliminate damaged cytoplasmic constituents including mis-folded proteins. It starts with the conversion of LC3, light chain 3, from its cytosolic form to a membrane-bound form, each named LC3 I and LC3 II. LC3 is a fluorescently tagged version of the mammalian homologue of yeast Atg8, which lapidated form functions as a key driver of autophagosome elongation and fusion. After LC3 is converted from LC3 I to II, the double membrane continues to elongate surrounding the target protein or organelle, until it encloses. The enclosed vehicle is called an autophagosome and eventually it binds with lysosome, forming autolysosome. Thus, LC3 is widely used as an important marker of autophagosome formation in mammalian studies. The contents of autolysosomes are broken down into smaller particles with acidic hydrolases that were previously present in the lysosome.

ii. Microautophagy

Microautophagy differs from macroautophagy as it does not involve the formation of autophagosome. This pathway is a non-selective process, which uses direct engulfment of cytoplasmic material into lysosome for degradation. The engulfment is called invagination, which means the inward folding of the lysosomal membrane, or cellular protrusion.[9] It is known to be induced by nitrogen starvation or rapamycin via regulatory signaling complex pathways. It functions to maintain organelle size, membrane homeostasis, and cell survival under nitrogen restriction.[10] Unlike macroautophagy, microautophagy is relatively less actively investigated.

iii. Chaperone-mediated Autophagy (CMA)

This type of autophagy is highly selective as it involves the recognition by the hsc70-containing complex, meaning that it can only work on proteins that include a recognition site for hsc70 complex. CMA substrate proteins are selectively targeted to lysosomes and translocated into the lysosomal lumen via coordinated action of chaperones located at both sides of the membrane and a dedicated protein translocation complex.[11] Hsc70 is found to not only function in targeting CMA substrate to the lysosomal membrane, where it can interact with the CMA receptor, but also in facilitating substrate unfolding that is crucial for the protein's translocation across the lysosomal

membrane.[12] One example of CMA is Parkin-mediated mitophagy. Parkin is one of the proteins that compose E3 ubiquitin ligase complex, which is essential in ubiquitin-proteasome system. Parkin-mediated mitophagy was found to be restored by metformin and suppressed by cytosolic p53 in study under Doctor Byung-Wan Lee. His article is discussed in the *pharmaceutical application of autophagy* section.

iv. Mitophagy

Mitophagy is a selective degradative mechanism that specifically function on damaged mitochondria. Mitochondria are important cellular components which regulate cellular energy homeostasis and cell death.[13] Controlling mitochondria count, mitophagy is a crucial mechanism for cell viability.[14] Mitophagy begins with the induction of general autophagy, but it includes an extra process of priming the damaged mitochondria for selective autophagic recognition, and this differs from other types of autophagy. This type of degradative mechanism was first observed in rat *hepatocytes*, liver cells, but its detailed pathways were documented and elucidated in yeast from its first examination in 2004. Studies show that mitophagy is controlled by Parkin and PTEN(phosphatase and tensin homolog)-induced putative kinase protein 1 (PINK1), and mutations in genes that codes these proteins have been linked to Parkinson's disease.[15]

d. Induction of Autophagy via AMPK Activation

As autophagy was proven to be a vital mechanism that helps maintain the body's homeostasis, researchers have paid great attention to discover methods to induce it. One mechanism of autophagy induction is via AMPK activation. Understanding the mechanism of AMPK activation in autophagy induction is essential in understanding how certain drugs induce autophagy and how it can be a cure for several diseases. AMPK, adenosine monophosphate (AMP)-activated protein kinase, is a critical energy sensor that regulates cellular metabolism to maintain energy homeostasis.[16] There are various pathways taken from AMPK activation to autophagy induction.

The simplest one is AMPK promoting autophagy directly by activating Ulk1 through phosphorylation of Ser 317 and Ser 777, observed under glucose starvation. Ulk1 is a mammalian serine/threonine protein kinase which is essential in early steps of autophagosome formation.[17] Another pathway is AMPK regulation inactivating TORC1 pathway under nutrient starvation. It is reported that TORC1 inhibition leads to autophagy activation by (1) phosphorylation and activation of TSC2 exchange factor that inactivation the Rheb GTPase, and (2) phosphorylation of Raptor.[18] Raptor is an mTOR-interacting partner[19]; mTOR is short for mammalian target of rapamycin, which is a central cell-growth regulator that integrates growth factors and nutrient signals. Phosphorylation of raptor leads to inhibition of mTOR which finally leads to autophagy. mTOR is active in nutrient sufficiency and hyper-

phosphorylates Atg13 that impede the formation of Atg13-Atg1 complex, preventing autophagy. On the other hand, mTOR is inhibited by starvation, which induce autophagy via dephosphorylation of Atg13 that binds to Atg1.[20] This creates a bigger complex with Atg 17, Atg29 and Atg 31. Formation of this complex lead to emergence of autophagosome in the cell.[21]

IV. Pharmaceutical Applications of Autophagy

Many drugs are created to cure specific diseases. However, there are several cases where new effects are discovered, like viagra. Viagra was originally developed to alleviate pulmonary hypertension, but was otherwise found to be effective to treat erectile dysfunction. In the same manner, this section introduces substances that were found to be related to autophagy induction in addition to their original function. Interviewees include those who have published their discoveries on the application of autophagy in pharmaceutical and medical use. This section is a case study on the researches done by Dr. Ho Jeong Kwon, professor of Yonsei University in College of Life Science and Biotechnology, and Dr. Byung-Wan Lee, professor of Yonsei University in College of Medicine.

a. Rapamycin

Rapamycin is the most popular autophagy inducer that is being commercialized. Rapamycin also functions as an immunosuppressant and an antiatherosclerotic,[22, 23] and it has been proved to be effective in preventing restenosis and organ transplantation rejection.[24] Rapamycin

activates autophagy by inhibiting mammalian target of rapamycin (mTOR), which is the major negative regulator of autophagy. One of important roles of mTOR is stimulating cell proliferation and cell growth. If mTOR is inhibited by rapamycin, it shows phenomenon related with cellular starvation and this eventually leads to autophagy.[25] Although many experimental results show mTOR suppression with rapamycin, it was not found effective in clinical trials.)[26, 27] Currently, rapamycin is actively used in laboratory studies for autophagy induction but was found to show no significant induction in mammalian cells.

b. Indatraline

Dr. Ho Jeong Kwon and his team (2016) announced their discovery on indatraline as an autophagy inducer. Indatraline, first developed as an anti-depressant, is a non-selective monoamine transporter inhibitor, which prevents the reabsorption of neurotransmitter. However, according to Dr. Kwon, indatraline also activates autophagy by AMPK/mTOR/S6K signaling transduction. More specifically, the paper states that indatraline decreases intracellular ATP level, increasing AMP/ATP ratio. As a trigger point of adenosine monophosphate-activated protein kinase (AMPK), increased AMP/ATP ratio activates AMPK and lead to the suppression of mTOR/S6 kinase signaling, which eventually stimulate the activation of autophagy.[24]

The significance of discovering indatraline as an autophagy inducer lies in the fact that currently used autophagy suppressor such as 3-MA and wortmannin cannot deactivate autophagy induced by indatraline. This implies that indatraline takes a different pathway to induce autophagy. Unlike rapamycin targeting mTOR signaling pathway, indatraline targets monoamine uptake receptor. Because this receptor is located in the cell membrane and mTOR in cytosol, monoamine uptake receptor can be considered as a higher-level signaling component than mTOR in controlling autophagy. Since indatraline utilizes a different pathway from that of rapamycin, Dr. Kwon explained that indatraline holds importance in being useful to subjects with high rapamycin-tolerance in autophagy induction. Another significant feature of indatraline is that it promotes autophagy independently from apoptosis whereas some other autophagy inducers such as rapamycin induce autophagy and apoptosis at the same time.[24] Synergistic or additive effects on cell death are likely to happen when autophagy and apoptosis occur simultaneously.[28]

c. Metformin

In similar context with indatraline, Dr. Byung-Wan Lee and his team found out that metformin, a popular drug used in diabetes treatment, can also be used as a mitophagy inducer, a type of autophagy that specifically degrades mitochondria. Further information on mitophagy can be found in *Types of Autophagy* section.

The paper introduces two methods of mitophagy induction using metformin: 1) *silent mating type information regulation 1* (SIRT1) and 2) AMPK targeting. Firstly, application of metformin activates SIRT1. SIRT1 is a deacetylase protein, which stimulates proteins that are essential in mitophagy induction like histones, p53, ATG5, ATG7, ATG8 and FoxO1. By stimulating such proteins, SIRT1 plays a role in mitophagy induction. Secondly, when AMPK is targeted, it induces mitophagy by activating AMPK which inhibits mTOR signaling. Once mTOR is impeded, the cell shows similar condition as to cellular starvation, leading to induction of autophagy.

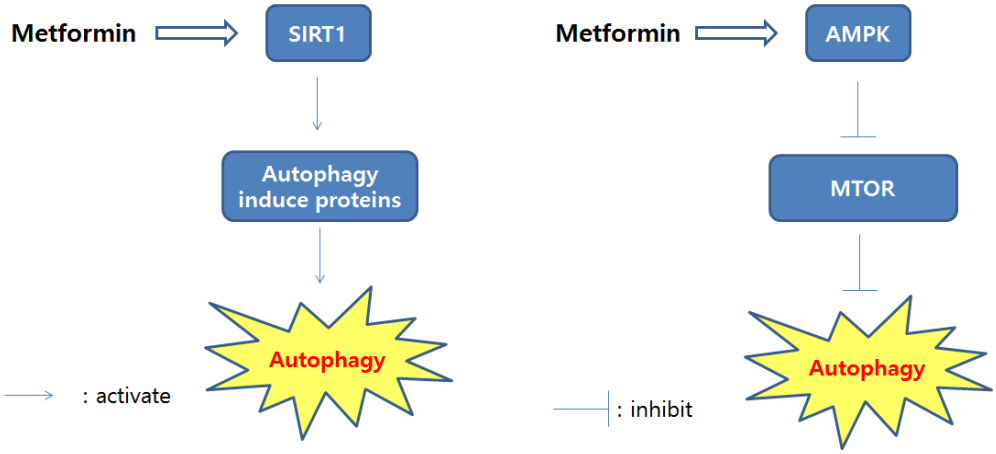

Figure 1. Metformin targeting SIRT1 (left) and AMPK (right)

Metformin-induced mitophagy is Parkin-mediated. More specifically, metformin suppresses the expression of endoplasmic reticulum (ER) and cytosolic p53. Though p53 is a tumor-suppressor protein, cytosolic p53 restrains autophagy activity by blocking mitochondrial translocation of Parkin, which leads to mitophagy

malfunction. Therefore, suppressing the expression of cytosolic p53 with metformin will induce Parkin-mediated mitophagy.[29]

V. Medical Applications of Autophagy

As autophagy was found to be an important cellular process, it has brought the attention of researchers in medical field to test its effectiveness in curing certain diseases. Being a degradative mechanism, it was found to be helpful in alleviating diseases caused by overproduction or insufficient clearing of several substances. The following section includes a case study of researches regarding the influence of autophagy induction in treatment of cardiovascular, internal, bone, and neurodegenerative diseases.

a. Cardiovascular (Restenosis/ Atherosclerosis)

Atherosclerosis (AS) is a common cardiovascular disease that is foreseen as one of the leading fatal disease worldwide in 2020.[30] Arteriosclerosis is a longer-term inflammatory disease of the arterial wall that is primarily caused by plaque and destabilization and rupture. It is caused by the narrowing of endothelium of the blood vessels due to accumulation of cholesterols and proliferation of endothelial cells. Current treatments for atherosclerosis include angioplasty, a blood vessel surgery, and stent placement, which is inserting metal or plastic tube to the blood vessel for expansion. The three types of initiation and development factors of atherosclerosis are 1) *macrophages*, 2) *smooth muscle cells* (SMCs), and 3) *vascular endothelial cells*.[30] Macrophages can cause atherosclerosis as they destabilize atherosclerotic plaque and

generate cholesterol flux. Therefore, atherosclerosis can be relieved via elimination of selective macrophage through autophagy. To induce autophagy as atherosclerosis treatment, rapamycin derivatives (sirolimus and everolimus) are currently in use[22, 31] as they inhibit proliferation of macrophages and plaque accumulation. However, this treatment was found to have risk of restenosis.[24]

Restenosis is "a narrowing of blood vessels caused by the rapid proliferation of smooth muscle cells (SMCs) or macrophages."[32] Angioplasty is currently used for restenosis treatment, but some studies shows that treatment through angioplasty had two times higher probability of restenosis occurance.[33] Hence, Dr. Kwon emphasized the necessity of developing restenosis-targeting therapies and introduced indatraline as an option. His paper includes data that show how autophagy induced by indatraline helps restrain SMC proliferation. Although rapamycin also inhibits neointimal hyperplasia caused by proliferation and migration of SMCs, rapamycin-induced autophagy is not apoptosis-independent cell death. On the other hand, indatraline is better in a way that it does not cause apoptosis.[24]

b. Internal (Diabetes/ Fatty Liver)

Diet heavily dependent on carbohydrate caused metabolic syndrome (MS) to be one of the most common diseases in 21st century. Metabolic syndrome includes diseases like fatty liver, hypertension, type 2 diabetes mellitus, atherosclerosis, myocardial infarction and vascular dementia. The major cause of this syndrome is insulin

resistance due to aging accompanied by abdominal obesity and visceral deposition of adipose tissue. Among mentioned illnesses, diabetes is known to cause high mortality rate due to its complications, and therefore has been an active research area. Diabetes is categorized into type 1 and 2. Type 2 diabetes is more common, and is caused by insulin resistance or insulinopenia (disorder in insulin secretion). As a result, insulin receptor's susceptibility in recognizing insulin decreases. Insulin resistance may be developed when low-grade inflammation consistently occurs because of lipid, reactive oxygen species, or ER stress.[34] For inflammation, activation of inflammasome is necessary. Inflammasome is a multiprotein oligomer expressed in myeloid cells. "Autophagy is a well-known modulator of inflammasome activation" and "autophagy regulates inflammasome activation by participating in the quality control of dysfunctional mitochondria".[35, 36] Therefore, Dr.Myung-Shik Lee, professor of Yonsei University college of Medicine, and his team had suggested that combination of excessive lipid accumulation and autophagy dysfunction causes metabolic syndrome or *inflammatory bowel syndrome (IBS)*, which leads to type 2 diabetes. According to Dr. Lee and his colleagues' experiment, autophagy deficiency in macrophage caused by lipid injury such as high fatty acids and cholesterol, brought inflammasome activation. This led low-grade inflammation which caused obesity-induced insulin resistance, in other words obesity-induced diabetes mellitus.[37]

As mentioned earlier, accumulation of visceral adipose tissue can bring serious consequences including metabolic syndrome. In

similar context, hepatocellular lipid accumulation also provokes fatty liver. A patient with fatty liver is likely to have hepatitis or chronic hepatitis and has a high chance of developing hepatocellular carcinoma. In 2012, Dr. Byung-Wan Lee and his colleges have found out that hepatocellular lipid accumulation can be reduced through autophagy that was induced by dimethyl sulfoxide (DMSO), a chemical chaperone. Since macroautophagy degrades not only misfolded and impaired proteins but also hepatocellular triglyceride accumulation, Dr. Lee also states that if autophagy is suppressed, it can lead to hepatosteatosis (fatty liver) because accumulated lipid droplets were not eliminated.[38]

c. Bone (Osteroporosis)

Diabetes is well known for its fatal complications. Osteoporosis is one of its complications which include weakened bone strength that can lead to bone fracture. In 2016, Dr. Wei-Lin Zhang, affiliated to the First Hospital of China Medical University, and his team had announced that autophagy suppression can be effective in treatment of type 2 diabetic osteoporosis. According to their report, autophagy "participates in the regulation of osteoblasts and osteoclasts, and has a close correlation with osteogenesis and bone absorption", causing negative effects on bones.[39, 40] Related to osteoporosis, Dr. Zhang's team had found out three different effects of melatonin on autophagy. Melatonin is a hormone generated and secreted in pineal gland and was found to have the following effects. Firstly, when melatonin was

injected to rat diabetes model it showed improvement in bone microstructure and decrease in autophagy level. Secondly, it enhanced osteogenesis and autophagy suppression in osteoblast that was cultured in high glucose level. High glucose level is known to stimulate the production of *reactive oxygen species (ROS)* which causes autophagy through ERK pathway activation[41, 42]; ERK pathway is also called RAS-RAF-MEK-ERK pathway.[43] Lastly, Melatonin was found to be able to function as an antioxidant that suppresses ROS production. Therefore, Dr.Zhang's team claimed that by suppressing ROS production with melatonin, autophagy in osteoblast can be reduced. Based on these findings, Dr.Zhang's team concluded that melatonin can inhibit autophagy in osteoblast and may "delay diabetes-induced osteoporosis by inhibiting ERK signaling pathway." [44]

d. Neurodegenerative Diseases (Alzheimer/ Autism)

Autophagosome is a double-membrane structure, formed as a phagophore encloses after gathering cytosolic wastes. In data analyses, production of autophagosome usually functions as an indicator of autophagy induction. However, healthy neurons do not contain many autophagosomes even when autophagy is working properly. This is because autophagy includes degradation of autophagosome in its activity. In 2011, Dr. Jun-Hua Liang and Dr. Jian-Ping Jia, from Xuan Wu Hospital of the Capital Medical University in Beijing, stated "neuronal autophagy is essential for neuronal survival and the maintenance of neuronal homeostasis". Lack of autophagosome is due

to autophagosome clearance is efficiently processed by basal autophagy. In neurons, autophagic flux, the net rate of autophagosome content degradation through the autophagic pathway is the indicator of autophagy efficiency.[45] Therefore in normal healthy brains, accumulation of *autophagic vacuoles (AV)* is hardly seen. On the other hand, *Alzheimer's disease* patients are known to have excessive amount of AVs in their brains. "The AVs accumulating in the AD brain are electron-dense autolysosomes and autophagosomes filled with undigested or incompletely-digested 'waste' proteins".[46] Since the amount of autophagosome reflects the "balance between AV formation and degradation", Dr. Liang and Jia suggest two possible causes of abnormal accumulation of autophagosome in neurons. First, contrary to active autophagy induction, digestive system in autophagy could be dysfunctional. Second possibility is, along with ineffective autophagosome production, autophagosome fusion and digestion with lysosome might not be fulfilled. In the case where autophagosome induction and autophagosome degradation are not efficiently operating, Dr. Liang and Jia proposed two kinds of therapies. Though currently there are some technical limits, Dr. Liang and Jia said "moderately increased levels of autophagic induction in combination with therapies to promote the successful completion of autophagic degradation might be promising intervention strategy". They also suggest using two different types of autophagy inducers that have different pathways to induce autophagy, for instance, rapamycin, which goes through mTOR pathway, and lithium, which has independent pathway with mTOR to

induce autophagy.[47, 48] They claim using different inducers that take separate pathways helps autophagy upregulation and eliminating protein aggregates.

However, treating Alzheimer's disease with autophagy still remains a little tricky because autophagy induction and inhibition are both necessary in the process of treatment. Dr. Liang and Jia's mouse experiment showed that autophagy induction can decrease neuronal aggregates substantially and delay the onset of neurological symptoms in AD model. Furthermore, using rapamycin as an autophagy inducer, Dr. Liang and Jia found that autophagy induction led to the reduction of amyloid plaques, *neurofibrillary tangles (NFTs)*, and cognitive deficits, which helps AD treatment.[49] On the other hand, autophagy inhibition is also important because not all induced autophagy functions effectively. If autophagosome degradation does not process properly, it generates *β-amyloid peptide (Aβ)* which brings higher chance of AD and enhances the exposure of catabolic substance in AVs. Furthermore, autophagy inhibition showed alleviation in Aβ42-induced cell death,[50, 51] when Aβ42 is known to promote and degenerate AD. Also, autophagy induction showed possibility in augmentation of Aβ1-42 neurotoxicity.[50] Therefore, Dr. Liang and Dr. Jia claimed that is it important to clearly define the model and stage of AD to maximize the use of autophagy induction in treatment. For example, autophagy induction showed positive results for AD treatment at the stage before development of AD-pathology in 3×Tg-AD mice. However, it showed no improvement after mature plaque

and tangle formation in AD. In Aβ42-expressing and Aβ40-expressing fly experimental models, autophagy inhibition through neuron-specific Atg5RNAi expression showed incompatible consequence. Unlike Aβ1-42 flies, Aβ40 flies lifespan have shortened, meaning "Aβ1-42 expression may shift protective neuronal autophagy to a pathogenic condition".[52]

Dr. Seung-Yong Yoon, professor of Ulsan University in College of Medicine, and his colleagues discovered that autophagy deficiency in microglia can cause *autism spectrum disorders (ASDs)*. According to Dr. Yoon's experiment, when a crucial autophagy gene called atg7 was deficient in microglia, it resulted in lack of synaptic refinement and abnormal neurobehavior regulation such as repetitive behavior and lack of social interaction ability. From these results, Dr. Yoon concluded that lack of autophagy results in reduced synaptic refinement, which finally lead to show symptoms of ASDs.[53]

e. Discussion on Pharmaceutical and Medical Application of Autophagy

Despite its potential effectiveness, there are limitations in autophagy application for pharmaceutical and medical purposes because lack of or too much of autophagy is usually not the main cause of illnesses. Inducing or inhibiting autophagy may alleviate such illnesses, but it is still difficult to conclude that autophagy can completely cure or prevent a disease. Rather, it will work as a supplementary treatment that goes side by side with major treatments. In their interviews, Dr. Byung-Wan Lee and Dr. S-Y Yoon both

explained that treatment using autophagy can be promising in the future, but autophagy inducers or inhibitors will not be used as the major treatment of diabetes or ASD. Furthermore, current technology and knowledge lack the ability to degrade specific target using autophagy and the development of more sophisticated technology is required to apply autophagy-modulating strategy for application in clinical applications. However, autophagy activation at certain target has been observed in some cases in various experiments.

Autophagy is a system that breaks down old and impaired cellular constituents and restores them. This characteristic creates a strong relationship with degenerative diseases such as Parkinson's disease and Alzheimer's disease. Including cancer, these degenerative diseases are currently popular research topics due to drastic increase in average life expectancy. In the near future, development of autophagy-related remedies will provide patients an extra option to treat their diseases without adverse reactions and invasive processes from surgeries.

VI. Cosmetic Applications of Autophagy

Skin anti-aging, whitening and prevention and curing of alopecia are concerns of many. Surprisingly, there already exists a few cosmetic companies that developed autophagy-inducing technologies and applied them to their products. Therefore, this paper intends to promote understanding of the mechanism of aging, melanogenesis (darkening) and hair loss along with few

of journals to explain its relationship with autophagy. Furthermore, it intends to introduce examples of autophagy related technology used in actual products.

a. Anti-aging and Autophagy

According to '*Skin Aging: New Research'*, "Skin aging is characterized by mild skin atrophy or deep rhytids, facial areas with muscle hypertrophy and changes in the distribution of subcutaneous tissue, telangiectasia, solar melanosis, poikiloderma and the development of premalignant and/or malignant skin lesions." It also states that skin aging is a complex process, thus even though many previous techniques and procedures intending to treat intrinsic and extrinsic skin aging were practiced, such as chemical peels and lasers, nothing has proven to succeed in preventing aging process of the skin.[54]

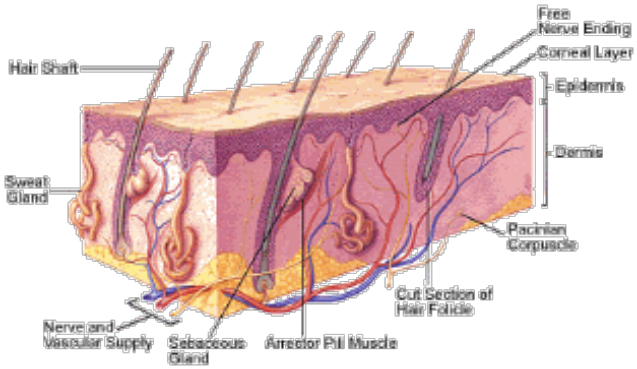

Figure 2. Anatomy of the skin [66]

Along with aging, epidermis layer of the skin becomes thinner, and the boundaries between epidermis and dermis become flatter. Therefore, surface of the skin is more easily damaged, in a way that it is more prone to form blisters and purple spots. Furthermore, healing

process is slower and the skin is generally drier. Melanocytes are known to decrease about 10% per year, which makes the skin vulnerable to UV light and the skin tone lighter. Even though the number of melanocytes decreases while the activation level of the skin cells are uneven, this leaves discolouration on the skin surface increasing the danger of forming malignant tumors. Furthermore, Langerhan's cells that are in charge of the immune system of the skin decreases in its functionality and number, which result in less active local immune system.

Generally speaking, dermis layer of the elders shows 20% decrease in size compared to younger age. This is because the regeneration of substrates in dermis layer decreases while the enzymes that breaks them down increases. Collagen in dermis, which composes about 80~85% of dermis, decreases and so as the elastin in its number and length. Also, hyaluronic acid level decreases in the mucopolysaccharides, decreasing the tension of the skin. This loss of tension is shown on the surface of the skin and usually results in wrinkles or drooped skin.[55]

Therefore, anti-aging of skin includes maintaining the thickness of epidermis and dermis layer of the skin. For example, by sustaining the level of keratinocytes, which composes 95% of epidermis layer[56], immune system on the surface of the skin against harmful external influences and the internal homeostasis are maintained. A recent research has shown the autophagy's correlation with keratinocyte that autophagy deficient keratinocytes display increased DNA damage,

senescence and aberrant lipid composition after oxidative stress in vitro and in vivo. According to this research, Atg7 deficient KC (Keratinocyte) displayed increased prostanoid signaling and a pro-mitotic gene expression compared to WT (Atg7 bearing cells). After exposure to PQ (paraquat), an oxidant drug commonly used to induce cellular senescence, both WT and KO (Atg7 deficient cells) showed an inflammatory and stress related transcriptomic response. KO has additionally showed drastic DNA damage and cell cycle arrest signaling. Furthermore, the absence of Atg7/autophagy resulted in disturbed lipid phenotype, which is, together with DNA damage and cell cycle arrest, all typical for premature cell aging.[57]

Figure 3. ageLOC R^2 products, Day and Night [67]

One of the companies utilizing anti-aging function of autophagy is Nu Skin. Nu Skin has developed a technology to preserve autophagy process through gene expression, and produced their product named ageLOC R^2. In their product information page of ageLOC R^2 Day and ageLOC R^2 Night, it is explained that "ageLOC science identifies, targets and resets Youth Gene Clusters (YGCs) to retune your youth-". It is also explained that ageLOC R^2 targets two important processes: cellular purification and cellular energy production.[58]

Though the specific mechanism of their technology is not disclosed, through purifying the accumulated damage, cellular waste and toxic byproducts with autophagy induction, ageLOC R^2 Night intends to slow down and reverse the aging process and recover cellular function that was lost because of aging. They have also emphasized that ageLOC R^2 is not focused on anti-aging of limited areas of the body, but rather of all the cells, "-helping you feel healthier, younger, and more vibrant than you have in years".[58]

Another area of focus that ageLOC R^2 aimed to activate is cellular energy production. Through understanding age-related vitality loss with decline in energy generation in our body, they have identified the problem of aging as decline in mitochondrial inefficiency and decrease in the number of mitochondria with age. In order to "fuel our bodies," ageLOC R^2 Day targets to promote mitochondrial activation through resetting YGC.

Answering to the question asking for any research proving the ability of ageLOC R^2, they said that "ageLOC R^2 ingredients were selected by comparing gene expression patterns to identify specific age-related gene expression changes connected with cellular energy production and cellular purification. Then, one specific set of functionally related genes were identified and dozens of natural compounds were screened in vivo in laboratory studies. Based on the screening process, we selected only the ingredients that modulated gene expression toward more youthful patterns."[58]

ageLOC R^2 is the first product to focus on the internal source of aging through application of autophagy, rather than the symptoms of the problem. This is an example of taking advantage of non-specific activation of autophagy throughout the whole body.

b. Whitening and Autophagy

Skin whitening has been at the center of attention for many Asian consumers due to admiration of white and fair skin promoted in the media. Scientific reports show that melanogenesis causes darkening of the skin: activated melanocytes synthesizing melanin.

Melanins are synthesized in the cells called *melanocytes*, specialized cells at the lowest level of epidermis, at the junction with upper dermal layer. Sun, stress, irritation and hormones usually trigger the activation of melanocytes, as it causes signal molecules to bind to the receptors on the surface of the mentioned cells. The binding activates an enzyme called *tyrosinase*, which then goes through a

series of reactions to synthesize melanin. First two steps require oxygen radicals, changing amino acid *tyrosine* into an intermediary molecule called DOPA (Dihydroxyphenylalanine). DOPA is then converted into secondary molecule called *dopaquinone*. These two steps are both catalyzed by an enzyme named *tyrosinase*. Then, Dopaquinone is converted into one of two types of melanin, *eumelanins* and *pheomelanins*, where *eumelanin* is the dominant pigment found in human skin. Once these melanins are synthesized, they are packed in membrane-bound packages called *melanosomes*. Nextly, these melanosomes moves towards the projecting arms of melanocytes and are transferred into adjacent keratinocytes. The melanosomes aggregate on the top of the nucleus of the keratinocyte and supply melanin to about thirty-six keratinocytes; this process lead to darkening of the skin.[59]

A recent study has shown involvement of autophagy in depigmentation; identified that depigmentation of α-melanocyte-stimulating hormone-treated melanoma cells by β- mangostin is mediated by selective autophagy. According to the paper published, "*β- mangostin has significantly inhibited the protein level of tyrosinase induced by α- MSH in UPS independent and lysosome dependent manner*", meaning that β- mangostin "*inhibits the melanogenesis induced by α-MSH via autophagy dependent mechanism*". Furthermore, unlike most of the autophagy related studies that involves non-selective autophagy, this paper suggests that

"*the depigmentation effect of β- mangostin may depend on autophagy targeted at the melanosome rather than non-selective autophagy*".[60]

Figure 4. Laneige White Plus Renew Original Essence advertisement[68]

One of the cosmetic companies that utilizes autophagy to develop a whitening technology is Laneige of Amore Pacific. Laneige has launched 'Laneige Original Essence White Plus Renew' in 2013, promoting its whitening technique called Melacrusher^{TM}, which recovers skin's melanin degradation abilities.[61] In Laneige's magazine, they reported their research on Asian consumer's demand for milky skin with healthy pink complexion, which is generally two tone brighter than their original skin color.[62] Laneige's 'Original Essence White Plus Renew' promises to unveil melanin layer of the skin. Their survey to 299 female in 5 Asian cities reports that 97.3% has experienced healthy whitening in two weeks with the use of their product. Laneige's promotion on their product has motion of delivering radiant skin with two tone whitening in '2' weeks with '4' free ingredients (non-Paraben, non-artificial colorants and mineral oil, non-animal originated ingredients), giving solutions to '6' whitening

concerns, which is luster, moisture, brightness, even skin tone, slowing melanin accumulation rate and reducing dullness.[63]

c. Alopecia and Autophagy

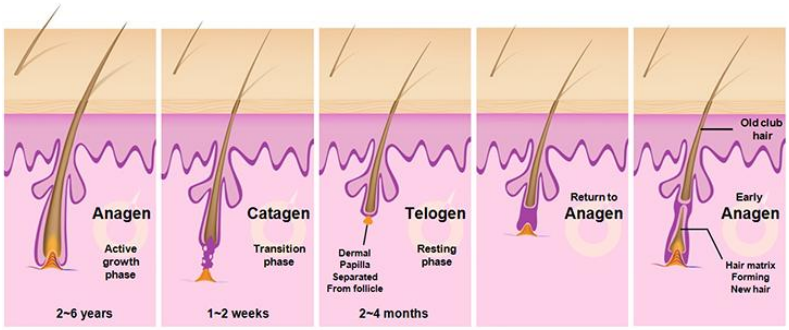

Figure 5. Hair growth phases, circulating from left to the right [69]

Hair growth can be divided into three phases, anagen phase, catagen phase and telogen phase. Anagen phase represents active hair development and production of hair fiber. This phase can last between two to six years. Therefore, healthy scalp holds 90% of its hair in its anagen phase. During catagen phase, hair follicles get smaller and rise up in the scalp. Hair is still attached to the follicle wall, but receives very little nourishment, lasting up to 3 weeks. Telogen phase is a resting phase, where dermal papilla is inactive and hair fibre and root sheaths stop growing. Therefore, hair easily falls out during brushing or washing. To some, however, telogen phase does not exist, with new hair growing immediately.[64]

Problems of hair growth in any of the stages explained above can lead to certain type of hair loss. Pathological causes are broad from infection, genetics to autoimmune diseases. Recent studies have also identified its relation to autophagy that lack in autophagy function can

lead to stunt growth of new hair. This study has confirmed significantly slow hair growth in Atg7-deficient mice, after the transplant of dorsal skin. Unlike the control group with mature hair bulbs with melanocytes, Atg7-deficient mice displayed a few immature hair bulbs. Furthermore, less amount of hair shafts were found in Atg7-deficient mice with thicker outer root sheath compared to that of control group.[65]

d. Future of Autophagy in Cosmetics

Considering the first discovery of autophagy being made only about a decade ago, its rapid application in commercial industries is astounding. As explained earlier, autophagy's commercial application in cosmetics has had its own progress especially in the last 5 years, developing industry's own technology, such as ageLOC and Melacrusher^{TM} technology. Yet, apart from autophagy application in skin whitening and anti-aging, other beauty concerns such as alopecia should be further studied and researched, finding its solution using autophagy.

However, with many of autophagy related techniques without complete autophagy pathway known, greater amount of research is certainly necessary for advancement in both commercial and academic fields. Discovery of autophagy's contribution in aging process and melanogenesis is essential for better understanding of autophagy itself and its reverse process, such as anti-aging and whitening. Better understanding of autophagy is expected to naturally help commercial

industries to have wider options of approaches that they can take, in order to meet the needs of their current and future consumers.

VII. Conclusion

The review of pharmaceutical applications of autophagy has shown the necessity to revisit existing medications to examine its function as an autophagy inducer or an inhibitor. Rapamycin, indatraline and metformin were examined for its potential as an autophagy inducer, thus imply other existing medication can also be withholding its function. Yet, discovered autophagy inducers mentioned above are currently in testing phases; possibility of them applied to actual treatment is still in question. However, with further research on autophagy regarding its specific induction and inhibition mechanisms, application of these pharmaceuticals to induce or inhibit autophagy is indeed possible.

From treating restenosis, atherosclerosis to bone fracture and neurodegenerative diseases, medical industries has high expectations on autophagy's potentials. Many diseases have shown improvement in healing or in its prevention when autophagy is induced compared to that of samples with blocked autophagy. Furthermore, autophagy induction enhanced regeneration process of new cells and prevented formation of tumors by cleaning out the cell's internal environment and maintaining homeostasis. Thus, autophagy carries potential of making our body younger, holding the possibility of bringing the solution to problems caused by aging of human body.

Autophagy's potential for anti-aging has already been noticed in cosmetic industries along with its role in skin whitening; autophagy related technologies have been developed, such as Melacrusher^{TM} and ageLOC R^2. Products made using these technologies are already available in the market. Apart from its impact on skin whitening and anti-aging, autophagy has also found to influence hair growth, thus can applied in treating alopecia.

Though the applications shown above has possibility of being applied to actual industries, many experts believe more developments should be made for autophagy-related technology to be widely and safely used in various industries. According to Professor Kwon, research on possibility of specific targeting is insufficient and so is the general understanding of autophagy mechanism, which makes clinical application in a short period of time problematic. Additionally, further research on excessive autophagy induction should be also considered for clinical safety, thus financial and human supports in research fields are necessary. Dr. Lee B.W. too, believes autophagy research to be clinically applied requires proof of it effectively treating or curing certain type of diseases. Therefore, if autophagy-related drugs or cure were to be clinically applied, it will be partially used or considered minor in complete treatment of the disease. Yet, since autophagy has its potential in anti-aging or cells, he is positive that its active research on neurodegenerative diseases can bring potential cure for diseases such as Alzheimer's. Similarly, Dr. Yoon mentioned the necessity of in-depth examination on autophagy mechanism in molecular level. By studying the autophagy relationship with diseases, specific pathological causes can be identified and targeted. Apart from future autophagy research heading towards

revealing its unknown molecular mechanism, Dr. Lee H.W. believes cooperative work of experts from different fields of research would create more significant results. Due to many existing variables of autophagy, countless possibilities need to be calculated and studied, thus it requires more than a single team or a small number of biologists working together.

It is difficult to say that there is a single cure for every illnesses Understanding of physiology of human body, pathology of diseases and so many other professional knowledge are required to cure an illness. The same applies to autophagy. Though so much research has been carried out since the discovery of autophagy,, complete elucidations of all autophagy mechanisms do not exist. This lack of understanding in basic mechanisms holds back application of autophagy in real-life industries, making it hard to target specific type of cells and identify the effect of autophagy induction and inhibition. With further research on these unknowns, however, the discovery of autophagy is giving possibility to treat diseases that was originally untreatable since it identifies the cause of the disease to be lack of autophagy. So far, we have considered one of the main causes of degenerative diseases to be aging. Diseases which come with age were often considered inevitable and incurable. Yet, anti-aging with autophagy gives silver lining to those degenerative diseases, improving quality of life.

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