A Bioinformatics Model Based on Autophagy Related Genes for Prognosis of Breast Cancer

A PROJECT REPORT

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Submitted by:

PROTISHA SEN
(2K21/BIO/03)

Under the supervision of
Dr.Asmita Das
(Assistant Professor)

DEPARTMENT OF BIOTECHNOLOGY
DELHI TECHNOLOGICAL UNIVERSITY
(Formerly Delhi College of Engineering)
Bawana Road, Delhi-110042

MAY, 2023

DELHI TECHNOLOGICAL UNIVERSITY
(Formerly Delhi College of Engineering)
Bawana Road, Delhi-110042

CANDIDATE'S DECLARATION

I, Protisha Sen, 2K21/BIO/03 of M.Tech (Bioinformatics), hereby declare that the project Dissertation titled **"A Bioinformatics Model based on Autophagy Related Genes for Prognosis of Breast Cancer"** which is submitted by me to the Department of Biotechnology, Delhi Technological University, Delhi in partial fulfilment of the requirements for the award of the degree of Master of Technology, is original and not copied from any source with proper citation. This work has not previously formed the basis for the award of the Degree, Diploma Associateship, Fellowship or other similar title or recognition.

Place: Delhi

Date: 29/05/2023

PROTISHA SEN

(2K21/BIO/03)

DEPARTMENT OF BIOTECHNOLOGY

DELHI TECHNOLOGICAL UNIVERSITY
Bawana Road,Delhi-110042

CERTIFICATE

I hereby certify that the Project Dissertation titled **"A Bioinformatics Model Based on Autophagy Related Genes for Prognosis of Breast Cancer"** which is submitted by **PROTISHA SEN**, Roll No.**2K21/BIO/03** Department of Biotechnology, Delhi Technological University, Delhi in partial fulfillment of the requirement for the award of degree of Master of Technology, is a record of the project work carried out by the student under my supervision. To the best of my knowledge, this work has not been submitted in part or full for any Degree or diploma to this University or elsewhere.

09/06/2023

(Prof. PRAVIR KUMAR)
HEAD OF THE DEPARTMENT

DEPARTMENT OF BIOTECHNOLOGY

(Dr. ASMITA DAS)
ASSISTANT PROFESSOR
SUPERVISOR
DEPARTMENT OF BIOTECHNOLOGY

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Protisha Sen
(2K21/BIO/03)
Dept. of Bio-Technology,
DTU, Delhi-110042

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A Bioinformatics Model based on Autophagy Related Genes for Prognosis of Breast Cancer

Protisha Sen
Delhi Technological University, Delhi, India

1. ABSTRACT

Autophagy is a biological catabolic process that controls self cleanliness within the cell. The cellular autophagy mechanism is aimed to remove senescent organelles and aberrant long-lived proteins from the body under normal physiological circumstances, which will be helpful for preserving cell homeostasis. A stress stimulus causes cell's autophagy to function, limiting the accumulation of toxic or cancer-causing damaged proteins and organelles while also reducing cell death. Therefore, autophagy malfunction has a significant effect on cell destiny and may play a role in carcinogenesis. Research has suggested that breast cancer cell autophagy and carcinogenesis are strongly connected. Breast CSCs are capable of self renewal & differentiation although showing favourable short term prognosis, however, cancer recurrence, chemotherapeutic resistance, and metastasis are quite common in long term. Reports show that metastatic breast cancer significantly boosts patient morbidity and mortality. Breast cancer metastasis is a very intricate process that is precisely regulated by a number of factors. Since it affects tumour dormancy, cancer stem cells, metabolic adaptability, cell motility, and migration in breast cancer, autophagy is one of the key regulatory elements regulating metastasis. Clinically, autophagy has been shown to be causative of therapeutic resistance and anti-estrogen treatment in breast cancer. Autophagy thus serves both as a promoter and a suppressor during the process of breast oncogenesis depending on the cancer stage. The aim of this bioinformatics study is to construct a novel model using the autophagy related gene signatures for prognosis of breast cancer in patients. These putative autophagy biomarkers could be evaluated in preclinical and clinical investigations to track the autophagy process in breast cancer therapy. It is possible to identify breast cancer patients who will respond to potent autophagy modulated therapy and have a favourable prognosis, although more investigation is required on this.

Key words: Autophagy, Homeostasis, Breast CSCs, Metastasis, Prognosis, Chemotherapeutic Resistance, Oncogenesis

2. INTRODUCTION

A number of stressful situations, such as organelle injury, the presence of abnormal senescent proteins, and nutritional scarcity, trigger autophagy, an intracellular degradative process.There are 3 categories of autophagy namely macro, micro and chaperone-mediated however, autophagy refers to "Macroautophagy". The process of autophagy starts with the production of autophagosomes, which then fuse with lysosomes to recycle destroyed components. Numerous clinical conditions, such as cardiomyopathy, muscle disorders, neurodegenerative conditions, and cancer, are linked to the dysregulation of autophagy. In many malignancies, the regulation of autophagy plays dual roles in tumour development and repression. Additionally, autophagy controls the stemness, recurrence, and resistance to anticancer drugs of cancer cells, all of which are characteristics of cancer stem cells.

Depending on the stage at which the cancer is progressing, cells are shielded from additional DNA damage and genomic instability during the early stages of cancer by autophagy-mediated clearance of malfunctioned cytosolic constituents, such as protein aggregation or damaged vesicles. In cancer cells with an apoptotic deficiency, autophagy can function as a cell-killing mechanism. Additionally, autophagy can promote tumour growth by facilitating oncogene-induced senescence or by defending tumours from necrosis and inflammation. Once cancer has developed, autophagy can aid tumour growth by enabling tumour cells to endure challenging circumstances. The hallmark of cancer, unrestricted proliferation, calls for plenty of nutrition and oxygen. Tumor cells with specific mutations depend on autophagy for survival, emphasizing the need to incorporate this concept into clinical trial design and carefully select cancer types or patients for autophagy therapies"[1]. It has a good role in protein secretion, immunogenicity regulation, and tumor cell invasion[2].Blocking autophagy has been shown to change the survival of proteins involved in carcinogenesis, secretion, proliferation, tumor editing, and invasion.

One of the most common malignancies in women is breast cancer. The median survival time for a sizable subset of patients is 18–30 months, and they are at high risk of metastasis. Patients with

metastatic breast cancer have a 5-year survival rate of about 20%. However, long-term survivors are still susceptible to recurrence, which might be accompanied by extremely aggressive metastases and resistance to the early treatments. This condition can be attributed to the considerable intratumoral heterogeneity of cell types present in breast tumours, which makes the creation of effective treatments more difficult [3]. Different expression profiles are linked to particular clinical characteristics according to the gene expression signature that divided breast tumours into subtypes. Four distinct intrinsic subtypes of breast cancer have been discovered through genomic studies: luminal A, luminal B, HER2-enriched, and triple-negative subgroups (Fig.1). Luminal A differs from luminal B in terms of their molecular expression profile. Breast cancer patients are clinically categorised based on the expression levels of the following molecular markers: the proliferation marker Ki-67, the estrogen receptor (ER), the progesterone receptor (PR), and the human epidermal growth factor receptor 2 (HER2 also known as ERBB2) [4,5,6,7]. Luminal malignancies include the ER positive category. When compared to Luminal A tumours, Luminal B tumours exhibit reduced ER or estrogen-regulated gene expression, little or no PR expression, higher tumour grade, higher expression of genes related to proliferation, and activation of growth factor receptor signalling, including the IGF-1R and PI3K/AKT/mTOR pathways. In addition to having a worse prognosis than the luminal subtypes, HER2-enriched breast tumours also express higher proliferation markers. Triple negative breast cancers (TNBC) is the most poorly prognosed breast cancer subtype, accounting for 10% of all patients [4,5,8,9,10]. These are mesenchymal, basal-like, and claudin-low tumours that have ER-/PR-/HER2- and frequently include mutations in the tumour suppressor genes BRCA1 and BRCA2, which are crucial for DNA repair. In malignant mammary cells, several ATGs may support tumor-suppressive characteristics. For instance, TNBC has been reported to have lower amounts of ATG7 protein than non-tumor tissue. High levels of ATG7 have been linked to increased overall survival in TNBC patients, and the expression of ATG7 reduced proliferation and glycolysis in TNBC cell lines [11].

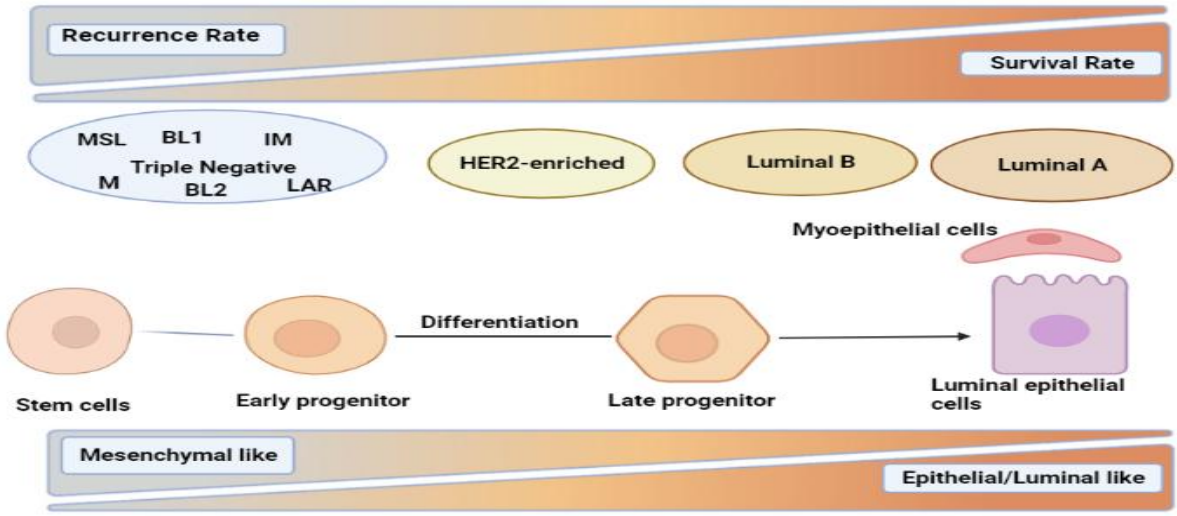


Fig.1 Four distinct intrinsic subtypes of breast cancer have been discovered through genomic studies: luminal A, luminal B, HER2-enriched, and triple-negative subgroups varying in the expression profiles of ER, PR, HER2 and Ki-67

Due to its cytoprotective function, autophagy is regarded as tumour suppressive in terms of the development of cancer. The elevated autophagy-related gene profile in healthy mammary glands is reduced as breast cancer progresses [12] (Figure 3), serves as proof of this. The first ATG protein whose absence was associated with breast cancer was beclin1, a part of the class III PI3K complex in the nucleation step of autophagy [13]. ATG proteins involved in the initiation complex, like WIPI1, WIPI2, and ULK1, as well as proteins involved in the production of autophagosomes, like ATG5, ATG10, ATG14, and GABARAP, were shown to be induced by increased expression of Forkhead Box O3 (FOXO3), according to findings. The absence of FOXO3 decreased the expression of several ATGs, which in turn decreased autophagic activity. As a result, the absence of FOXO3 caused the development of mammary tumours, indicating that the loss of FOXO3-mediated autophagy may result in mammtary carcinogenesis [14,15,16].

Metastases are either directly or indirectly responsible for 90% of breast cancer fatalities. By assisting cancer cells in surviving extracellular matrix separation, one of the initial steps in metastasis development and cancer cell migration, autophagy further contributes to the metastatic process [17]. Autophagy, however, reduces prometastatic differentiation and metastatic expansion in murine breast cancer models at later phases of the metastatic process.

The autophagy cargo receptor NBR1 and p62/SQSTM1 can accumulate as a result of impaired autophagy in circulating tumour cells. These aggregated cargo receptors can operate as signalling scaffolds to trigger the nuclear factor erythroid 2-related factor 2 (NRF2) cell survival pathway or the Mitogen Associated Protein Kinase (MAPK) pathway. [18,19,20].Therefore, there is an overall need for breast cancer metastatic predictors exists. Although, it is believed that autophagy is a tumor-suppressive process. However, once a tumour has developed, it might help tumour cells survive in response to therapy or metabolic stress. However, it has also been proposed that autophagy might be activated during breast cancer treatment to eliminate cells that resist apoptosis. Thus, through this bioinformatics analysis, it would be possible for us to consider autophagy as a treatment pathway for Breast Cancer.

3. REVIEW OF LITERATURE

3.1 Mechanism Of Autophagy

Autophagy or cell's process of self- cleaning is a natural, subcellular degradation mechanism that breaks down undesired cargo such as damaged or aged organelles, undesirable proteins, and pathogens before releasing the digested macromolecules back into the cytosol. "Induction, vesicle nucleation, elongation, docking, fusion, breakdown, and recycling comprise the macroautophagic process" (Figure 2)[21]. Autophagy being a trafficking mechanism is heavily influenced by the environmental conditions such as invoked in absence of nutrition and certain diseases or activated in response to specific hormones in mammalian cells.Autophagy was first explained in 1963 by Christian de Duve [21] which involves the sequestration of cell organelles and cytoplasmic material into double-membrane vesicles called autophagosomes before delivering them to the lysosomes for lysosomal hydrolase-mediated degradation [22][23][24].

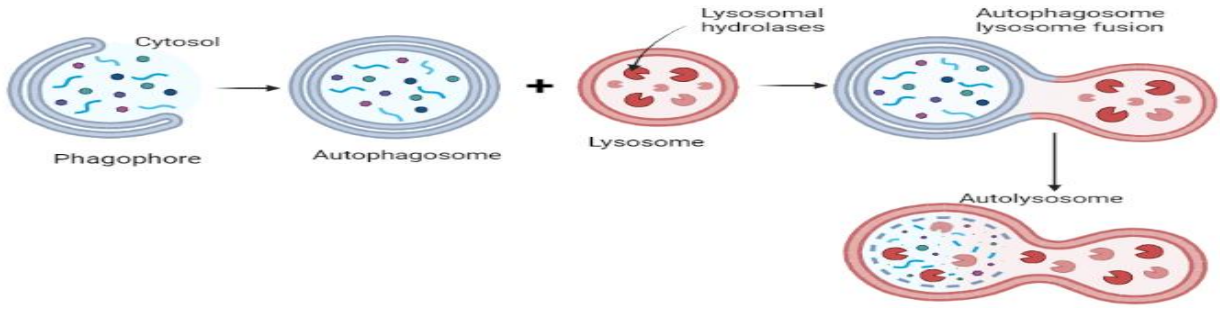


Fig.2 Steps in the "Macroautophagic" Process

Parts of the cytoplasm are captured during autophagy in a double-membrane autophagosome and transported to a degradative organelle—the lysosome in mammalian cells or in a vacuole in yeast for degradation and subsequent recycling of the resultant macromolecules. The cell is freed from numerous stress-related circumstances by this mechanism. Autophagy is essential for cellular growth and differentiation, suppresses tumour growth in early phases, and may even help cells live longer. Moreover, autophagy has a variety of roles in both innate and adaptive immunity, including defence against pathogen invasion.

In eukaryotes, autophagy is carried out through three distinct pathways: macroautophagy, microautophagy, and chaperone-mediated autophagy. The lysosomal breakdown of cellular cargo occurs at the end of all these three mechanistically distinct pathways. The encapsulation of cellular cargo into double-membrane vesicles known as autophagosomes, which is the hallmark of macroautophagy, is a feature shared by all eukaryotes. In yeast, autophagy-related (Atg) proteins which are recruited hierarchically to the phagophore assembly site or the pre-autophagosomal structure (PAS) are responsible for the formation of autophagosomes around the targeted cargo.Two complexes are required to commence the autophagosome formation process. (1) A complex made up of the class III PI3 K Vps34, Atg6/Beclin1, Atg14, and Vps15/p150.73 molecules.(2) The serine/threonine kinase Atg1 is a component of the other complex. The de novo synthesis of the double membrane structure phagophore or also known as an isolation membrane, whose lipid constituents are derived from the golgi-endosome system, is made possible at the pre-autophagosomal structure (PAS) by initiator protein complexes [24][25]. Several cellular organelles, including the plasma membrane, are known to act as origins for the formation of a phagophore in mammals, where a specific PAS-like structure has not been found yet [26].The isolation membrane becomes elongated into a phagophore with the recruitment of additional Atg protein complexes,the Atg8/MAP-LC3/GABARAP/GATE-16 and Atg12 systems. The phagophore eventually fuses at its free ends to create an autophagosome, which now encloses and sequesters the cargo.The Atg16-Atg5-Atg12 complex separates from its surrounding membrane as soon as the autophagosome is formed, and its component parts participate in the recycling process of the cargo mediated by Atg2, Atg9, and Atg18 [27]. When they move through the endocytic pathway after being generated, autophagosomes go through a maturation phase before joining with lysosomes to form autophagolysosomes. The hydrolytic enzymes of the lysosomes subsequently break down the cellular cargo that the autophagosomes

have delivered, and the resulting byproducts are released back into the cytoplasm for utilization by the cell [26][24].

The development of an autophagosome is not necessary for the other two autophagy mechanisms. In chaperone-mediated autophagy, certain chaperone proteins bind to the cargo and carry it across the lysosomal membrane for breakdown [28]. In microautophagy, invaginations or protrusions of the lysosomal membrane are employed to capture the cargo protein [29].Organelles in their whole are up taken at the lysosome's limiting membrane here.

3.2 Role of Autophagy in Cancer: The “Double-Edged Barrel”

Abnormalities in the intracellular process of autophagy can lead to neurodegenerative conditions, ageing and various types of cancers. During the early stages of cancer, autophagy hinders the development of tumours, but during the more advanced stages, it encourages tumour progression defining it’s cellular mechanism to be a “double-edged” barrel. Additionally, autophagy shields the tumour from many treatments by giving the cancer cells nutrients and energy from recycling & degradation of components. Tumour suppressor proteins encourage autophagy, whereas oncogenes prevent it. Autophagy offers great potential for the development of new and effective cancer therapies as well as the treatment of chemoresistant malignancies because of its changing bipolar function as per the stage of cancer.

Tumor Suppressor

Autophagic mechanism when interrupted can encourage and hasten the development of tumors. Studies have shown that it often regulates oncogene expression and tumor suppressor proteins.Protein kinases like mTOR and AMPK control tumor suppressors or other autophagy factors results in tumor suppression. mTOR signalling causes autophagic cell death and prevents stomach cancer cells from metastasizing [30]. Cancer cells promote cell development by stopping cells from breaking down damaged proteins or other components under oxidative stress (Fig.3). In multiple studies, basal autophagy inhibits cancer. By eliminating damaged organelles and proteins, autophagy controls cell proliferation and prevents genetic instability to decrease tumors [31]. Beclin 1 is a protein needed to carry out the process of autophagy and since as per experiments mutated Beclin 1+/-organisms were shown to be tumor-prone, Beclin 1 is considered to be a tumor suppressor gene [32]. When autophagy-related genes like Beclin 1 and

LC3 are silenced, breast cancer cells are less likely to proliferate, migrate, invade, and eventually succumb to apoptosis [32]. Collectively, these results show that autophagy plays a role in tumor suppression. According to one study, inhibiting mTOR signalling causes autophagic cell death and prevents stomach cancer cells from metastasizing [33].Another crucial role of autophagy is the elimination of cellular waste products collected as unfolded proteins, damaged organelles, and high-cargo receptor p62 in response to metabolic stress during tumour growth.Accumulation of p62/SQSTM 1 protein aggregates, damaged mitochondria, and misfolded proteins that result in the formation of reactive oxygen species (ROS) are possible molecular mechanisms linking faulty autophagy and cancer. Collectively, these results show that autophagy plays a role in tumor suppression.

Tumour Promoter

Several RAS-activated tumor types, including lung, pancreatic tumors, have enhanced baseline autophagy. Autophagy inhibition may promote tumor cell proliferation and development in such malignancies. Recent research shows that even in the presence of abundant nutrients, human cancer cell lines with activating mutations in H-ras or K-ras have high basal levels of autophagy [34]. Hypoxia and food deprivation are common to tumours. Cells are able to handle these pressures due to cell survival mechanism aided by autophagy. Elevated basal levels of autophagy were found in human pancreatic cancer cell lines and tumour tissues, and it was demonstrated that these levels support cellular energy production and promote tumour cell proliferation[35]. . Autophagy facilitates the invasion and epithelial-mesenchymal transition (EMT) of hepatocellular carcinoma cells in response to nutrient deprivation [36]. Cell death is accelerated by Beclin 1 deletion, which suppresses autophagy [37,38].The maintenance and tumorigenicity of breast cancer stem cells depend on Beclin-1 expression and subsequent autophagy activation. While the silencing of the autophagy-related gene, ATG12, was reported to lower the tumour cells' ability to invade in an organotypic model of glioma cells, the autophagy-associated factors DRAM1 and p62 have been found to regulate the energy metabolism and invasion of glioma stem cells through activation of autophagy [39, 40].By enhancing the ability of cancer cells to migrate and invade, autophagy speeds up the course of cancer.

Via its control of CD24 expression and IL6 release, autophagy also plays a part in the survival of cancer stem cells . In the MCF7 and MDA-MB-468 breast cancer models, autophagy-deficient

cells can form mammospheres again when given IL6 or treated with conditioned media from autophagy competent cells, indicating that autophagy is necessary for IL6 to be secreted in order to sustain cancer stem cells [41]. The maintenance of cancer stem cells' pluripotency also depends on basal autophagy, and any deviation from this level, whether through activation or inhibition, may induce differentiation and senescence [42].

Activation of autophagy in cancer cells that survive chemotherapy and/or radiation may allow for a state of dormancy in remaining cancer cells, which may aid in tumour development and recurrence [43]. It has been demonstrated that preventing autophagy in tumour cells increases the effectiveness of anticancer medications. Exposure to external stimuli, disease stage, and the tumour microenvironment all have a significant role to play in the activity of autophagy in cancer. To fully understand autophagy's therapeutic potential as a cancer treatment target, greater research is necessary into its contentious function in cancer.

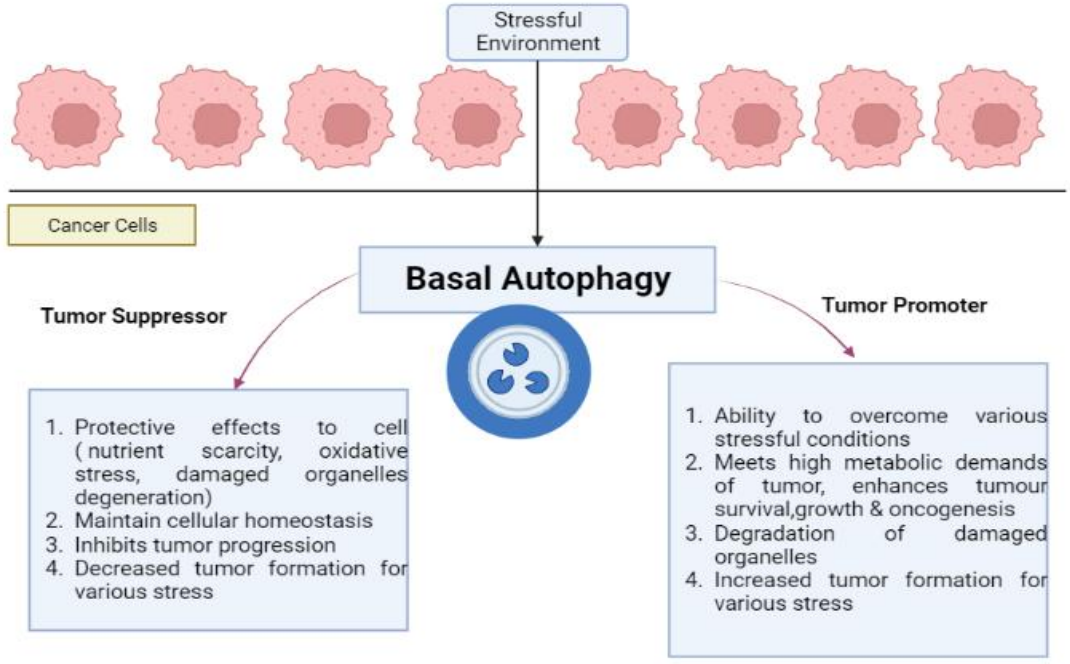


Fig.3 Autophagy dual role: Tumor Promoter and Tumor Suppressor

Autophagy in Pre-metastatic Vs Post-metastatic Stage

A more detailed research on the role of autophagy in cancer progression revealed that autophagy supports a number of processes in the metastatic cascade. Autophagy plays a key role in different events of metastatic cell invasion, intravasation, tumor circulation, extravasation, tumor survival, and secondary-site tumor growth (Fig.4). It regulates metastatic growth pathways, including focal adhesion, integrin trafficking, cytoskeleton remodeling, anoikis resistance, extracellular matrix dissociation, EMT, and tumor-stromal contact.

LC3 indicators link metastasis to autophagy flux in several cancers. As recently demonstrated, "metastasis proteins affect autophagy". Stress-activated nuclear protein -1 (NUPR1) enhances breast, pancreatic, brain, and thyroid metastasis. NUPR1 counteract with doxorubicin-induced genotoxic pressure, has a more complex role, including autophagy. BAG3 has a vital role in autophagy, apoptosis, cell adhesion, stress response, angiogenesis, and autophagy flux. BAG3, HSP70, and LC3 are autophagy polyubiquitinated proteins[44]. Tumor exosomes aid in interacting with neighbouring cells in the tumor micro-environment , which accelerate metastatic spread. Proteins, mRNAs, microRNAs, lipids, and soluble factors includes growth factors, cytokines, and integrins contained in the exosomes are transported to the surrounding cells. Exosomes released by metastatic melanoma cells reprogram bone marrow progenitor cells to be pro-vasculogenic and pro-metastatic, causing vascular leakiness in pre-metastatic sites. Renal carcinoma CSCs release exosomes that stimulate lung tumors and normal endothelial cells. Endolysosomal membrane system autophagy impacts tumor cells exosome production and may form pre-metastatic habitats[45].

Autophagy has been shown in past studies to play a key role in cancer cells dormancy required at the secondary site as they help them stay dormant longer, generating recurrent tumors by letting them survive in metabolic stress and hypoxia environment as well as by eliminating mitochondria, changing redox balance, and boosting CSCs and also make them therapy resistant. Dormant, scattered cancer cells may survive for years before producing lethal metastatic tumors. UPR(Unfolded protein response) -induced autophagy in dormant cells may also help in tumor survival[46]. Disseminated tumour cells (DTCs) use autophagy to support inactive and quiescent cell survival upon initial seeding of distant metastatic locations. Autophagy can prevent the formation of aggressive subpopulations of tumour cells with a high proliferation potential as DTCs enter a proliferative growth phase. Finally, MHC-I, which is essential for immunological identification of tumour cells, is selectively destroyed by autophagy. A role in creating the pre-

metastatic niche, boosting tumor cell survival, evading immune surveillance, and other elements necessary to eventually develop an overt metastasis are only just some of the recently identified activities for autophagy in metastasis.

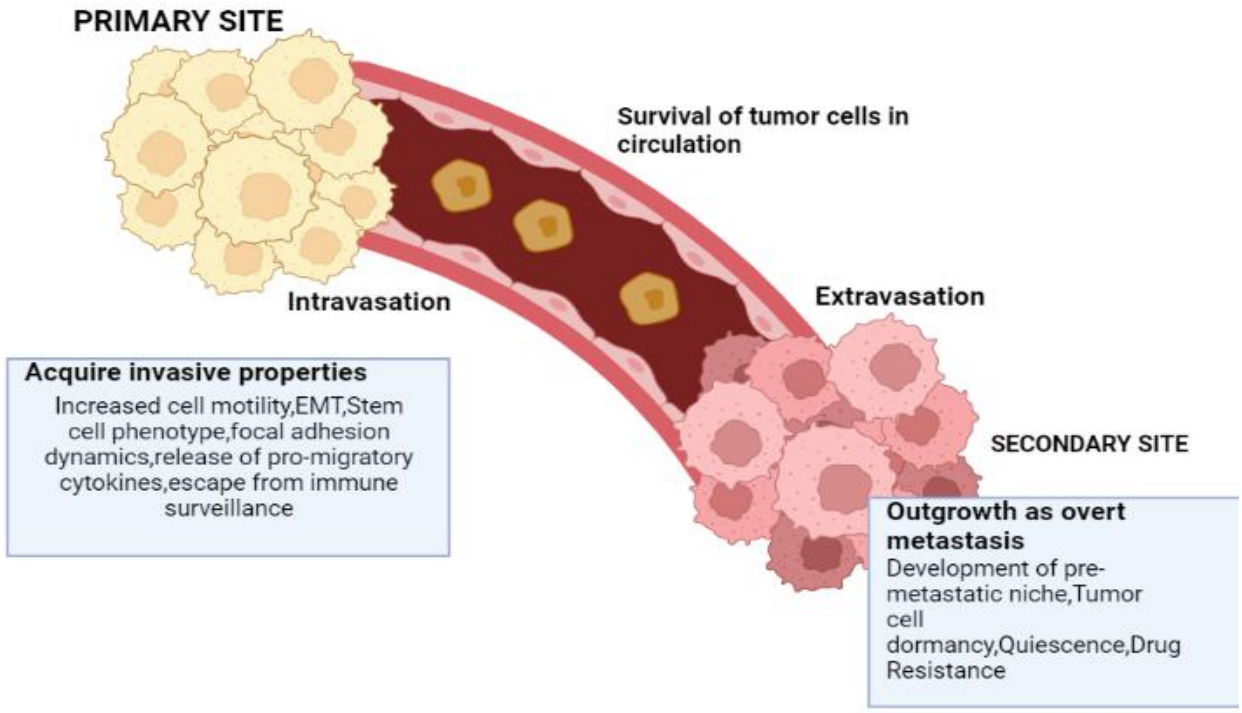


Fig.4 Autophagy in Metastatic Stages

3.3 Breast Cancer

Breast cancer develops when cells in your breast multiply and multiply out of control, resulting in a mass of tissue known as a tumour. Breast cancer symptoms can include witnessing a lump, noticing a change in breast size, or noticing changes to the skin around your breasts. Breast cancer can spread to the tissue surrounding your breast, just as other types of cancer and develop new tumours. There are three basic components of a breast: connective tissue, ducts, and lobules (Fig.5). The glands that generate milk are called lobules. Milk travels through tubes called ducts to the nipple. The connective tissue, which is made up of fatty and fibrous tissue, envelops and holds everything in place. The ducts or lobules are where most breast cancers start. Depending on the region, breast cancer can be divided into following subtypes:

- **Invasive ductal carcinoma** The cancer cells begin in the ducts and then grow outside the ducts into other parts of the breast tissue. Invasive cancer cells can also spread, or metastasize, to other parts of the body.

- **Invasive lobular carcinoma** Cancer cells begin in the lobules and then spread from the lobules to the breast tissues that are close by.
- **Ductal carcinoma in situ** Ductal carcinoma in situ, also known as Stage 0 breast cancer, is regarded sometimes as precancerous because the cells haven't moved past the milk ducts.
- **Lobular carcinoma in situ** Breast lobules with abnormal cells are referred to as lobular carcinoma in situ. Although it isn't an actual cancer, this indication may point to a later risk of breast cancer. Therefore, it's crucial for women with lobular carcinoma in situ to get routine mammograms and clinical breast exams.
- **Inflammatory breast cancer** This kind of cancer is uncommon and severe. Redness, inflammation, creasing of the breast skin are typical symptoms of inflammatory breast cancer. It is brought about by obstructive cancer cells in the lymphatic vessels under the skin.
- **Paget's disease of the breast** The nipple's skin and the skin around it, known as the areola, are both affected by this malignancy.

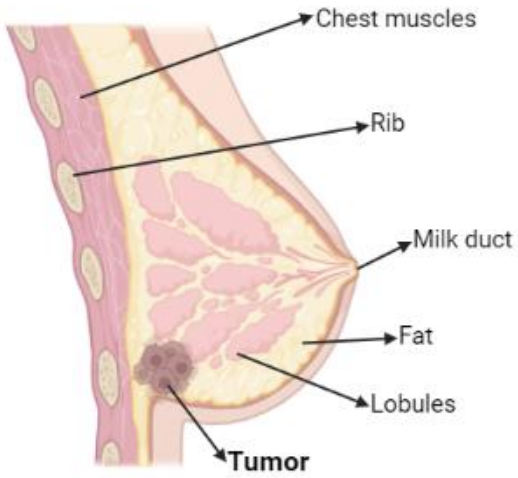


Fig.5 Diseased Breast Tissue

Surgery, hormone therapy, immunotherapy,radiation therapy, chemotherapy and targeted medication therapy comprise the breast cancer treatments available. The location and size of the tumour, the outcomes of lab tests, and if the cancer has spread to other regions of the body decide the most effective treatment for the patient. The treatment plan is designed to

meet the specific and individual needs of the patient. Receiving a combination of different treatments is also a significant concept nowdays in therapeutics.

Breast cancer has three primary subtypes, which are identified by performing particular tests on a sample of the tumour to ascertain its characteristics.The tumour sample can be examined to determine the type of cancer:

- **HER2 positive** The human epidermal growth factor receptor 2 (HER2) gene is required for the growth of 14% to 21% of breast tumours. These malignancies are referred to as "HER2 positive" and have a lot of HER2 gene copies or increased levels of HER2 protein. These proteins are also called "receptors." The HER2 protein, which is produced by the HER2 gene and is present on cancer cells, is crucial for the development of tumor cells. The tumor can be HER2 positive or HER2 negative. Breast tumours that are HER2-positive spread more quickly. HER2-targeted treatments are frequently used to treat early-stage breast cancers that are HER2-positive.
- **Hormone receptor positive** Breast tumours that express ER and/or PR are referred to as "hormone receptor positive." Cells have these receptors, which are proteins. "ER positive" refers to tumours that express estrogen receptors. Those tumours that express progesterone receptors are referred to be "PR positive." For a tumour to be referred to as hormone receptor positive, only 1 of these receptors needed to be positive. These receptors are absent in cancers, which are referred to as "hormone receptor negative." Hormone therapy is frequently used to treat hormone receptor positive breast cancers.
- **Triple negative** A tumour is referred to as "triple negative" if it lacks the expression of ER, PR, and HER2. Between 11% and 22% of invasive breast cancers are triple-negative. Women who have a BRCA1 gene mutation are also more likely to develop triple-negative breast cancer.

3.4 Link Between Autophagy and Breast Cancer

Since the gene beclin 1 was discovered to be deleted in 40%–75% of sporadic human breast and ovarian cancers, the theory that beclin 1 functions as a tumour suppressor was especially relevant for breast cancer. Beclin 1 expression is usually high in normal breast epithelia and low in

human breast epithelial cancer tissues.BRCA1, a tumour suppressor gene whose deletion contributes to various breast and ovarian malignancies, is located adjacent to beclin 1. The loss of beclin 1 in breast and ovarian cancers is consistent with the fact that BRCA1 deletion is the predominant driving mutation in these malignancies, and recent research suggests that beclin 1 itself may not be a tumour suppressor in this situation[47]. In addition to this, studies imply that the interaction between the two proteins, beclin 1 and bcl-2, may be particularly significant for the development of breast cancer tumours since a decrease in beclin 1 levels or its deletion would result in an increase in free bcl-2 and an antiapoptotic response. Additionally, it has been demonstrated that Bcl-2's ability to adhere to beclin 1 and block autophagy corresponds with its growth-promoting activity rather than its anti-apoptotic role, promoting tumorigenesis[48].

In another study, researchers made use of mice lacking Palb2 in the mammary gland, which resulted in invasive tumors and had DNA damage, breaks in DNA and p53 alterations. Since allelic loss of beclin1 had no effect on tumour formation when p53 was also removed from the mammary gland, it was hypothesised that autophagy was being activated in response to DNA damage and oxidative stress and mediated survival of tumour cells in conjunction with p53[49]. In oncogene-driven breast cancer models, autophagy was revealed to have a tumor-promoting function in the investigation, suggesting that autophagy addiction may be an acceptable therapeutic target in breast cancer.

Significant carcinogenic disturbances like RAS transformation or changes in the RAS pathway, which cause changes in metabolic pathways to meet biosynthetic needs, have been specifically associated to autophagy dependency. Active oncogene pathways like HER2, Myc, and active PI3K induce metabolic changes that are similar to RAS transformation even though RAS transformation is uncommon in breast cancer. Additionally, basal-like malignancies have increased PI3K and RAS-RAF-MEK pathway components, and RAS transformation in breast cancer cells can cause autophagy addiction[50].

Following matrix detachment, autophagy is activated in non-transformed and oncogene-transformed breast cell lines, shielding them from programmed cell death. The majority of breast cancer tumors would almost certainly exhibit changes in the autophagic system because mutations frequently found in breast malignancies are known to be crucial regulators of this route. This is especially true for Bcl-2, EGFR, p53, PI3K mutations, and changes to the PI3K-mTOR pathway[51].

3.5 Autophagy in Breast Cancer Progression

Elevated levels of autophagy are required for healthy mammary cell development to shield the cells from multiple multiple metabolic stresses. On the other hand, low levels of autophagy are necessary for the growth of malignant tumors from tumor-initiating cells, while high levels of autophagy maintain tumor cells dormancy by protecting against stressful conditions (Fig.6). Parallel to this, decreased autophagic activity stabilises Pfkfb3, which advances the cell cycle and prevents apoptosis. As a result, the growth of benign mammaries requires high autophagic activity, whereas the development of malignant mammaries requires low autophagic activity. Due to its cytoprotective function, autophagy is regarded as tumour suppressive in the initial stages of cancer. An elevated autophagy-related gene profile in healthy mammary glands, which disappears as breast cancer progresses, serves as proof of this.

Apart from loss of the component Beclin 1 activity in class III PI3K complex involved in the nucleation step of autophagy leading to normal cells being transformed to tumorous breast cells , there are other autophagy related factors as well aiding in breast cancer progression. Deletion of the crucial early autophagy protein FIP200 led to autophagy defects like protein aggregate accumulation and dysfunctional mitochondria as well as slowed the growth and progression of mammary tumors in a mouse model of PyMT-induced breast cancer [52]. As a result, suppressing MAP1LC3 or BECN1 decreased the expression of cyclin D1, integrin-1, and phosphorylated proto-oncogene tyrosine-protein kinase (SRC), which promotes entry into the G1 phase of the cell cycle and aids in the initiation and progression of breast cancer [53]. The autophagy cargo receptor NBR1 and p62/SQSTM1 can accumulate as a result of impaired autophagy in circulating tumour cells. The Nuclear factor erythroid 2-related factor 2 (NRF2) or Mitogen-Associated Protein Kinase (MAPK) pathways for cell survival can both be activated by these aggregated cargo receptors.

Overall, the evidence shows that autophagy that maintains cellular integrity. However, depending on stage of tumor, the ATG proteins may also promote breast tumorigenesis and cancer progression.

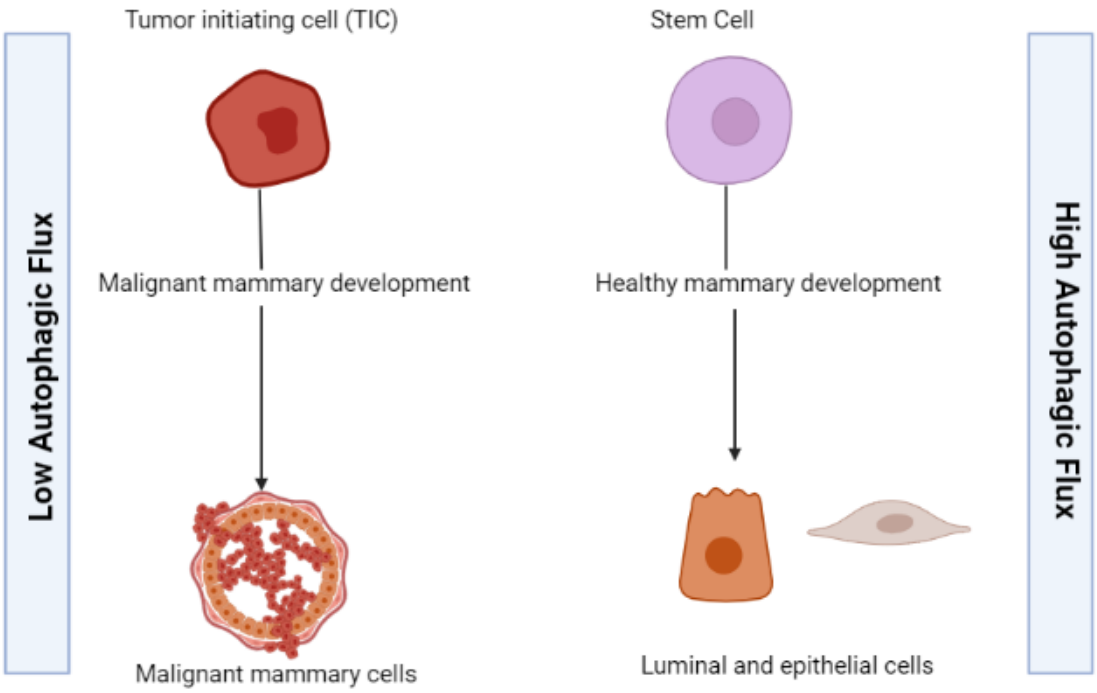


Fig.6 Autophagy in Healthy Breast Cells development VS Malignant Mammary Cells

3.6 Autophagy and Breast Cancer Stem Cells

Breast cancer stem cells (BCSCs), which may develop into a variety of cell types similar to normal mammary stem cells, are the primary cause of tumour formation in malignant breast tissue.The recurrence of tumors and metastases may be caused by CSCs, which would impact the course of treatment [54]. Aldehyde dehydrogenase (ALDH), CD44, and CD24 are the three primary markers of CSC connection with breast cancer [55]. Different breast cancer molecular subtypes exhibit different CSC markers, such as ALDH, CD44, or CD24. ALDH is crucial for maintaining stem cells and therapy resistance along with catalysing the oxidation of aldehydes. Also, triple negative breast tumors have a poor prognosis because CD44+/CD24-/low cells predominate in such tumors [56].

The proliferation and pluripotency of BCSC were decreased by pharmacologically blocking autophagy or by silencing BECN1, ATG7, or ATG4A [24]. The CD44high/CD24low BCSC population was decreased when MAP1LC3 or ATG12 was knocked down in HER2-enriched breast cancer cells [57].

Interleukin 6 (IL6), which is necessary for the upkeep of BCSC and the activation of the oncogenic STAT3 signalling pathway, is secreted in TNBCs facilitated by autophagy. Transforming growth factor beta 2 (Tgfβ2) and transforming growth factor beta 3 (Tgfβ3) mRNA levels also decreased when autophagic activity was reduced, which eliminated SMAD signalling, which is necessary for CD29HighCD61+ BCSCs. Thus, via the IL6/STAT3 and Epidermal Growth Factor Receptor (EGFR)/STAT3 as well as TGFβ/SMAD pathways, autophagy promotes BCSC maintenance. It's interesting to note that, depending on the subtype of breast cancer, autophagy lowers IL6 secretion, which may reduce BCSC counts [58]. Autophagy can also hinder BCSC by causing apoptosis in exceptional cases.However, this may depend on the cellular environment and the molecular background of the BCSCs.

The activity of CSCs can be maintained during anticancer therapy due to autophagy, which can facilitate tumour cell development and can result in resistance to the effects of the treatment medications. Recent studies have shown that the impacts of CSCs can be reduced by deactivating genes related to autophagy in order to limit autophagy (Fig. 7). This strategy can be combined with traditional cancer treatment methods to treat cancer more effectively.

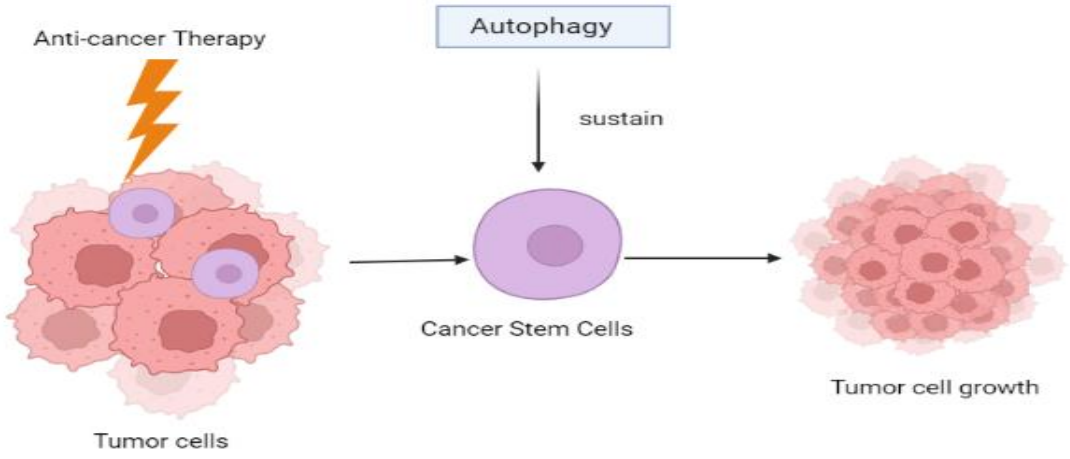


Fig.7 Autophagy and Anti-cancer Therapy Resistance of Breast CSCs

3.7 Autophagy in Breast Cancer Metastasis

The primary factor in deaths related to cancer is metastasis, which is the spread of cancer cells from the primary tumour to other secondary sites of the body. Insufficient amounts of nutrients and oxygen favour the metastasis of cancer cells. The physical transfer of cancer cells from a primary site to a distant location and the colonisation at the metastatic site are the two steps of

the breast cancer metastatic process. Invasion and migration of the tumor cells are the most important steps of cancer progression. Increased autophagy and metastasis have been found to be closely related via research using intermediary markers. While melanoma metastases showed greater LC3B staining compared with matched primary tumour samples, enhanced microtubule-associated light chain B (LC3B) punctae staining has been linked to lymph node metastasis and poor survival in human breast cancer [59]. Autophagy and metastasis interact in a complicated manner. EMT (epithelial-to-mesenchymal transition) is crucial for the dissemination and spread of cancer. By stabilizing Twist1, autophagy deficiency can induce EMT [60]. For instance, chaperone-regulated autophagy was found to increase breast cancer cell metastasis by downregulating macroautophagy related with ATG5 (61).

In a study , Marsh et al. (2020) used cell lines from mouse breast tumours that were modified to allow for the inducing removal of autophagy in order to study the impact of autophagy on breast cancer metastasis. By genetically inactivating the autophagy genes Atg5 or Atg12 in these cells, the researchers were able to inhibit autophagy in the cells after injecting them into the systemic circulation of mice. Results showed that compared to autophagy-competent cells, metastases produced by autophagy-deficient breast cancer cells were much larger and contained more proliferative cells. Breast cancer cells with impaired autophagy were also better able to metastasize from primary tumours. These findings suggest that autophagy limits the metastatic expansion of dispersed breast cancer cells, in contrast to its function in original tumours.

3.8 Autophagy in Breast Cancer Cell Dissemination and Dormancy

Disseminating cells which are a part of the metastatic cascade have been found to be leading cause of breast cancer-related fatalities [62]. Epithelial to Mesenchymal Transition (EMT) responsible for providing mesenchymal properties to the cells is significant in cell dissemination. The chemokine IL6 regulated by autophagy, which is particularly abundant in mammary tissue and is abundantly expressed in adipocytes, is a key regulator of EMT in breast cancer cells. Through the Janus Kinase (JAK)/STAT and MAPK signalling pathways, IL6 promotes EMT. Indicating that autophagic activity in breast cancer cells and in cells from the tumour microenvironment, consisting of adipocytes or immune cells, influences EMT via IL6/STAT and IL6 MAPK signalling [63].

Other facts include silencing of ATG7 or ATG12 caused an increase in focal adhesion complex(FAC) size in breast cancer cells, which is accompanied by a decrease in migration rate, proving evidence that autophagy inhibited migration through stabilising FAC. Additionally, destabilising the integrin 1 signalling pathway by silencing MAP1LC3 or BECN1 inhibited autophagy by inhibiting the activation of SRC and the Urokinase-Type Plasminogen Activator (uPA) system, which are crucial mediators of cell migration and invasion [64].When considered as a whole, autophagy is crucial to migration-related processes such as FA turnover and EMT. Therefore, autophagy is crucial for breast cancer cells as they migrate through the body. However, the circumstances and breast cancer subtype play a role in determining whether autophagy promotes or inhibits migration.

At early-stages of breast cancer ,disseminated tumour cells can be found, which can remain dormant for decades before becoming proliferative in response to changes in the microenvironment that activate autophagy [65]. Disseminated tumor cells are required to remain dormant at secondary site to fight stressful conditions making them anti-cancer therapy resistant, metastatic and reason for disease relapse. In response to environmental stress circumstances like food deficiency, it has been stated that autophagy is increased during extravasation and colonisation at distant locales [66]. Breast cancer cells secrete auto- and paracrine signalling molecules to prevent the activation of phosphoinositide 3-kinase (PI3K) under unfavourable circumstances. Inhibiting AKT and mTOR (the mammalian target of rapamycin) as a result causes the cell to enter a dormant state by activating autophagy. Furthermore, by triggering the autophagic pathway, ATG4, ATG7, and the ATG8 homologs maintain metabolic balance in dormant breast cancer cells. In particular, reducing mitophagy in dormant metastatic breast cancer cells by using HCQ or by knocking out ATG7 reduced autophagy, which resulted in the accumulation of damaged mitochondria and ROS, which decreased the survival of dormant breast cancer cells and prevented the switch from dormancy to growth [67]. In conclusion, these results unambiguously show that autophagy promotes dormancy in breast cancer cells.

3.9 Autophagy and Apoptosis in Breast Cancer

Apoptosis being programmed cell death is a common cellular response to metabolic stress and essential for limiting carcinogenesis in tumor cells in particular (Fig.8). The idea that apoptosis is suppressed in many tumors by a variety of mechanisms, such as overexpression of the apoptosis inhibitor BCL2, as well as the knowledge that preventing apoptotic cell death enables tumor cells

to survive the stress of oncogene activation, unchecked proliferation, and chemotherapy. In fact, BCL2 antagonists have been approved for use in the clinic as a tool to functionally reactivate the apoptotic pathway in refractory tumors.

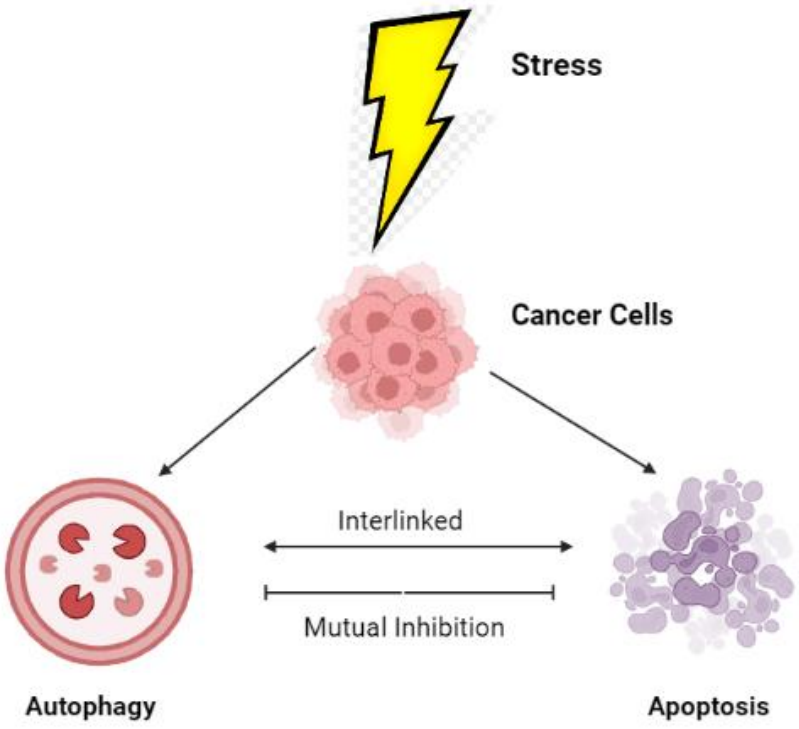


Fig.8 Tumor Suppressive role of Autophagy and Apoptosis in Cancer

The receptor pathway and the mitochondrial pathway are the two distinct pathways that can mediate apoptosis. It has been suggested that DAPK, a protein linked to death, may not be present in many cancer cell types. Beclin1 can be released from Bcl-2-related proteins as a result of phosphorylation, which also triggers autophagy. Bcl-2, a protein that caspases breaks, is an antagonistic regulator of Beclin1. This protein has the ability to suppress autophagy and promote apoptosis. Calpains break down ATG5 to create an N-terminal ATG5 cleavage product that is thought to trigger the release of cytochrome c from mitochondria. By controlling the autophagic breakdown of active caspase-8, Beclin1 and ATG5 block this process (68). This implies that the same regulatory elements may be involved in both autophagy and apoptosis (Fig. 9).

Furthermore, a caspase-dependent, apoptotic response to DNA damage was revealed by downregulating the autophagy proteins Beclin 1 and Atg7. It is suggested that early

autophagosomal removal of damaged mitochondria causes a post-mitochondrial caspase cascade to be delayed. Findings also imply that non benign breast carcinoma cells may have a tendency to use autophagy to delay apoptosis or increase survival. These results highlight the possibility of using autophagy inhibitors in combination with traditional chemotherapeutic agents to treat early breast cancer.

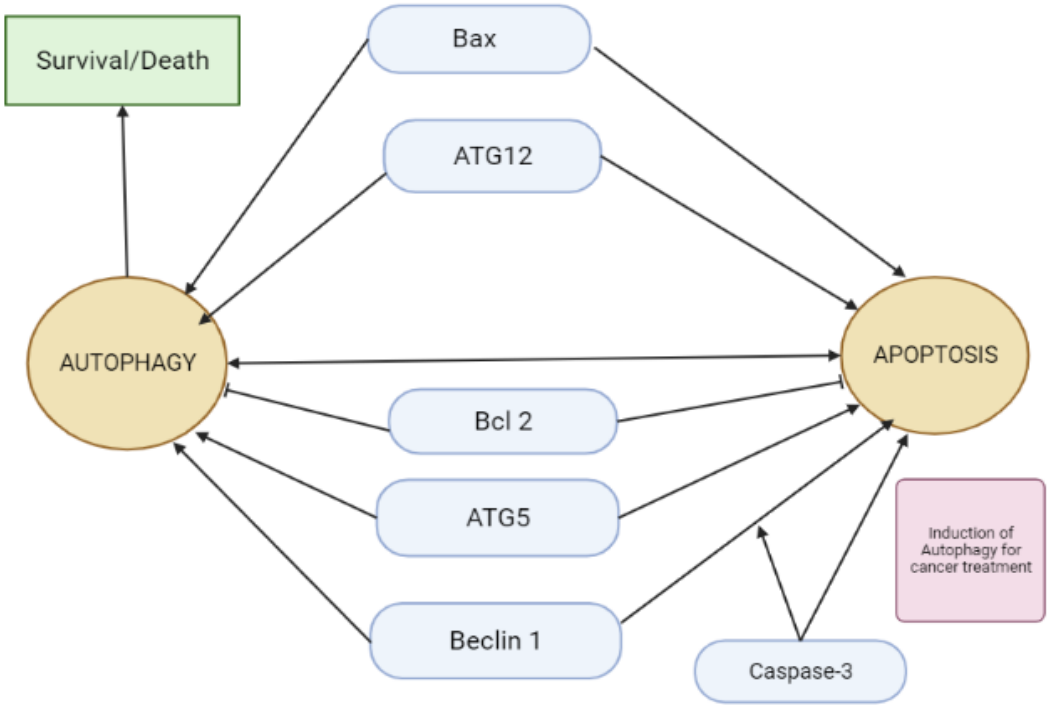


Fig.9 Regulatory elements in Autophagy and Apoptosis

Both autophagy and apoptosis are important mechanisms in the incredibly complicated processes of cell death and survival. Lu et al. found that parthenolide-induced apoptosis in breast cancer was improved by the suppression of autophagy (69). So autophagy and apoptosis are closely related to one another. Understanding the processes of autophagy in breast cancer and CSCs depends on the examination of this interlink.

3.10 Autophagy related Signaling Pathways and Molecules in Breast Cancer

ATGs regulate autophagy, which is a crucial factor in both breast cancer and breast CSCs. To enhance the efficacy of breast cancer therapy, further understanding of the functions of ATGs may be necessary. The primary autophagy regulatory gene Beclin1 expression is elevated in gastric cancer, colorectal cancer, cervical cancer, breast cancer, and liver cancer, indicating that

autophagy may be involved in carcinogenesis and that Beclin1 is an important factor in tumor development.

P53 is a familiar cancer suppressor protein that primarily inhibits cancer by inducing autophagy based on the expression of genes associated to autophagy, inhibiting PI3K/AKT/mTOR, and limiting EMT based on decreased expression of ZEB1, ZEB2, and SNAIL. mTOR is a evolutionarily conserved serine-threonine kinase that regulates a number of mammalian cell activity including cell development, metabolism, and proliferation.Rates of transcription, translation, protein degradation, cytoskeleton dynamics, cell metabolism, and autophagy are all considerably affected by changes in mTOR activity. Early research has shown that the mTOR inhibitor rapamycin may cause autophagy. According to a prior study, blocking the mTOR pathway alone in patients with HER2-positive cancer may trigger autophagy, allowing cancer cells to escape and develop treatment resistance [70]. To block the progression of cancer, it is therefore required to study the inhibition of other signal transduction pathways, dual pathway inhibition or multiple pathway inhibition.

The regulation of autophagy can be brought about by SMAD2/SMAD3 through the action of transforming growth factor TGF-β. Autophagy and the Wnt signalling pathway are related, and autophagy may be induced when the Wnt signalling system is inhibited. p62/SQSTM1 expression and the development of autophagic vacuoles can both be suppressed by β-catenin via transcription factor 4 (TCF4) (71). Hedgehog pathway inhibition has the same results as Wnt and Notch pathway inhibition, according to research (72).

Following are some molecules have the ability to control breast CSCs and breast cancer (Table1)

Molecules	Functions	
	Breast cancer	Breast CSCs
mTOR	With an increased amount of protein synthesis and increased inhibition of autophagy, the activation of mTOR complexes can promote the growth of tumors. Research has generally demonstrated that activated mTOR signaling increases tumor progression and often decreases patient survival. High mTOR expression is associated with poor prognosis in patients with breast cancer	Increasingly, research has connected mTOR activity to a phenotype of stem cell-like cancer, which can mediate breast cancer metastasis. In addition, studies have confirmed that the inhibition of mTORC1/2 can spare a cell population with stem cell-like properties and facilitate Notch activity in triple-negative breast cancer cell lines
ULK1	Expression of the kinase-dead ULK1 mutant K46N induced by hypoxia inhibits autophagy and increases lung metastases in MDA-MB-231 xenograft tumor mouse models. Similarly, the expression of the dominant-negative mutant of ULK1, ATG4b or ULK1-shRNA enhances cell migration *in vitro*	Targeting AMPK-ULK1-mediated autophagy can combat resistance to BET inhibitor in acute myeloid leukemia stem cells, which demonstrates that ULK1-mediated autophagy can maintain the activity of stem cells
FIP200	FIP200, as a component of the ULK1-ATG13-FIP200-ATG101 complex, has been demonstrated to be important for autophagosome formation. FIP200 deficiency can decrease tumor initiation and progression by disrupting tumor cell proliferation and increasing immune surveillance in an MMTV-PyMT mouse model of human breast cancer	Deletion of FIP200 (*Rb1cc1*) decreases tumor development and progression in MMTV-PyMT-driven mammary tumors and does not affect the percentage of $ALDH^+$ breast CSCs. However, it significantly reduces the $CD29^{hi}CD61^+$ breast cancer CSCs in comparison with isogenic (vehicle-treated) control cells

3.11 Inhibition of Autophagy in Breast Cancer

Breast CSC activity and the anticancer resistance response of breast cancer cells may both be safeguarded by autophagy. Additionally, autophagy can have an impact on the development, growth, and evolution of tumors and CSCs. Autophagy suppression may accelerate the demise of cancer cells. Therefore, it's critical to expand our understanding of autophagy inhibitors (Table II). Breast cancer therapies must be understood in relation to these autophagy inhibitors in current stage of research . These substances have the potential to inhibit autophagy, which may be important for discovering new treatment strategies.

Compounds such as eriocalyxin B (73), tetrandrine (74) and suberoylanilide hydroxamic acid, which is also essential in the treatment of breast cancer (75), can have an impact on autophagy. Trastuzumab and the autophagy/lysosome inhibitor chloroquine were also used to increase cell death via apoptosis in cells in vitro and in vivo, decrease cell survival, and hinder colony formation [76]. According to our data, the majority of autophagy inhibitors might be crucial in both breast cancer and breast CSCs. To find out whether 3-methyladeninecan or chloroquine can directly stop breast cancer cell growth, more investigation is needed. Therefore, it is important to pay more attention to these medications' new therapeutic mechanisms.

Table 2 Inhibitors of Autophagy in Cancer

FUNCTIONS		
	Compounds	Breast Cancer
1.	Mefloquine	Mefloquine suppresses autophagy and triggers the death of breast cancer cells.
2.	Quinacrine	Quinacrine prevents the acidification of lysosomes. Quinacrine effectively eliminates cancer cells and prevents their growth.
3.	Chloroquine (CQ)	CQ has an impact on the fusion of autophagosomes and lysosomes, which can prevent autophagy. CQ and other

		anticancer medications together may accelerate the apoptosis of breast cancer cells.
4.	Salinomycin	Salinomycin causes breast cancer cells to go into apoptosis.
5.	3-Methyladenine (3-MA)	Autophagosome creation is prevented by 3-MA. Increased apoptosis of breast cancer cells is possible when 3-MA is combined with other anticancer medications.

3.11 Targeting Autophagy in Breast Cancer Therapy

Particular significance is given to the function of autophagy and chemoresistance in the treatment of breast cancer. The stage of the disease and previous treatment regimen have a significant impact on how autophagy regulation affects the therapeutics of breast cancer. In tumour cells, autophagy is typically activated as a stress response to a particular anti-cancer treatment. Commonly used chemotherapy medicines can cause excessive or long-lasting autophagy, which causes cancer cells to die in a process known as autophagic cell death. To increase the cytotoxic effect in this situation, drugs that can increase autophagy levels (such as new mTORC1/2 kinases, Bcl-2 or HDAC-selective inhibitors, etc.) may be used. Contrarily, induced autophagy during chemotherapy may have cytoprotective effects that cause drug resistance and cause tumour recurrence.

Adjuvant endocrine therapy lowers the death rate from breast cancer, although many ER+ tumours become resistant and eventually relapse. Autophagy has been proposed to have a role in endocrine therapy resistance mechanisms, despite the fact that these mechanisms are poorly understood[77]. In the MCF7 breast cancer cell line, autophagy is triggered in response to anti-estrogen therapy, and its suppression makes the cell line more sensitive to tamoxifen treatment[78]. Additionally, it has been demonstrated that co-inhibition of Bcl-2 and BCL2L2 by preventing autophagy sensitises an anti-estrogen resistant cell line derived from MCF7 to the restoration of antiestrogen sensitivity.

It is known that genotoxic stress brought on by chemotherapy or ionising radiation raises p53 levels and causes cell cycle arrest, apoptosis, senescence, or autophagy[79]. In this respect, it has

recently been established that p53 plays two distinct roles depending on where it is located within the cell. P53 can suppress autophagy in the cytoplasm and is both transcription dependent and independent pro-autophagic in the nucleus[80]. Therefore, it is not surprising that radiation and DNA-damaging drugs used in treatment might cause autophagy in breast cancer cell lines[81–82].

Although the majority of the evidence outlined above suggests that autophagy should be suppressed to enhance breast cancer therapy, some studies also raise the possibility that autophagy may be responsible for the cell's demise, implying that autophagy may occasionally be increased during breast cancer treatment. For instance, it has been demonstrated that the deletion of Bcl-2 in MCF7 cells causes autophagy and non-apoptotic cell death, which was reduced by the deletion of ATG5, indicating that autophagy may be a factor in cell death in this model[82].

4. PROGNOSIS OF BREAST CANCER

The WHO Global Breast Cancer Initiative (GBCI) aims to prevent 2.6 million breast cancer deaths worldwide between 2020 and 2040 by reducing the annual global breast cancer mortality rate by 2.6%. 23% of breast cancer deaths among women under the age of 65 would be avoided by 2030 and 38% by 2040 if the global rate of breast cancer mortality was reduced by 2.5% annually[84]. Early detection methods, prompt diagnosis, and comprehensive breast cancer management are the three foundations for achieving these goals. A prognosis is the medical practitioner's best prediction of your cancer's effects and how it will react to therapy. The percentage of patients with an illness who are still living after being diagnosed is termed as survival. Numerous factors affect prognosis and survival.

Doctors can assess a patient's likelihood of surviving breast cancer using a variety of statistics. These are referred to as survival rates. The percentage of persons who are still living after receiving a diagnosis of breast cancer, is shown by the overall survival rate. For instance ,80% of women with stage I breast cancer survived the disease overall for five years. According to this, 80% of women with stage I breast cancer survive for at least 5 years after their initial diagnosis. The majority of these women would survive far above their diagnoses' five-year mark. The stage of breast cancer affects overall survival rates. Breast cancer patients who are diagnosed in stages 0, I, or II often have better overall survival rates than those who are diagnosed in stages III or IV.

The relative survival rate is a particular kind of survival statistic. It is frequently used to forecast potential life expectancy effects of cancer. The relative survival rate examines the likelihood that someone with breast cancer would live a specific amount of time following their first diagnosis or the beginning of therapy in comparison to the anticipated survival of individuals with similar characteristics who do not have this cancer.

There are numerous independent but connected prognostic variables that can predict survival and recurrence in breast cancer. These include oncogene amplification,tumor type, tumour size, age of diagnosis, axillary nodal status, histology, steroid receptors, proliferative rate, and ploidy[2]. The conventional gold standard indicator for predicting survival and recurrence in primary breast cancer has been axillary nodal status. Additionally, longer disease-free intervals and overall survival in stage I and stage II breast cancer have been linked to the presence of the estrogen and progesterone receptors. Indicators of cell proliferation that correlate with the relapse rate in breast cancer patients in pre- and postmenopausal women are the thymidine-labeling index. Stage I recurrence is more likely in oestrogen and progesterone receptor-negative tumours because they are more frequently aneuploid and have a larger percentage of S-phase.

Online tools can be utilised by doctors to determine prognosis of breast cancer.

Prognosis Programs The programmes combine data from significant research papers with information about the individual and their breast cancer. The findings are frequently displayed as a percentage survival rate at five and/or 10 to 12 years after diagnosis in the form of graphs. Some programmes also calculate the improvement in survival brought on by therapies like hormone therapy or chemotherapy. In order to assist you in deciding whether to receive these therapies, the doctor might display a graph of this data to the patient.

Nottingham Prognostic Index (NPI) This scoring method considers the size and grade of the breast cancer as well as whether any breast cancer cells are present in the lymph nodes. The patient's prognosis is given a score that ranges from good to bad. Estimates of the number of individuals still alive five years following diagnosis are provided for each group.

Genomic assays (Gene Expression Profiling) To determine the likelihood of recurrence, certain tests evaluate collection of genes identified in breast cancer. They are not appropriate for everyone and are often only taken into consideration in case of invasive breast cancer, oestrogen receptor positive (ER+), HER2 negative, and no more than three positive lymph nodes.

Predict Predict is an online tool for making decisions. It makes estimates about how various treatments following surgery for early invasive breast cancer might increase survival using information about the patient and her breast cancer.**Prosigna** For individuals who will be undergoing hormone therapy for at least five years, this test forecasts the likelihood that a malignancy may metastasize to another area of the body within ten years. Scores on the test range from 0 to 100. The results are classified as 'low', 'mid', or 'high' risk depending on this score and whether any lymph nodes under the arm are involved. The score, together with other details regarding breast cancer, will be used by the specialist to assist choose the best course of action.

Endopredict For individuals who will be undergoing hormone therapy for at least five years, this test forecasts the likelihood that a malignancy may metastasize to another area of the body within ten years. The outcome, known as the EPclin score, indicates whether the risk is high or low. A low risk score indicates that the breast cancer is not expected to spread over the following ten years. Chemotherapy is typically ineffective for those with low risk scores. A high risk score indicates a higher likelihood that the breast cancer will spread over the following ten years. For the majority of persons with a high risk score, chemotherapy is advised.

Oncotype DX This test forecasts the likelihood that a cancer will return following surgery as well as the anticipated benefits of receiving treatment.

5. OBJECTIVE

(a)To construct a model based on autophagy related genes using Bioinformatics for prognosis of Breast Cancer

(b)To conduct survival analysis statistics on the resultant autophagy related genes

6.METHODOLOGY

In this study, we aimed to investigate the role of autophagy-related genes in the prognosis of breast cancer. Differential gene expression analysis, gene set enrichment analysis (GSEA), Cox regression, as well as survival analysis were all part of our complete technique, which also made use of publicly available gene expression datasets. Initially, we identified suitable gene expression datasets from public repositories, specifically focusing on datasets with sufficient sample size, breast cancer subtypes of interest, and availability of survival data. The selected datasets were downloaded and pre-processed, including necessary normalization and quality control steps. We next applied statistical criteria, including adjusted p-value and fold change, to data from breast cancer samples and normal tissue controls in order to use the GEO2R programme to identify differentially expressed genes (DEGs). To focus on autophagy-related genes, we filtered the DEG list to retain genes that were annotated or implicated in autophagy pathways.

We used GSEA to determine whether or not the detected DEGs were enriched for gene sets linked to autophagy. Established autophagy gene sets from publicly accessible databases and published literature were used for this analysis. Enrichment scores were calculated, and statistical significance was evaluated using permutation tests or false discovery rate (FDR) correction. We used Cox regression analysis to determine whether or not there was a correlation between gene expression and survival. The chosen datasets were mined for survival information, such as overall survival as well as disease-free survival rates. Autophagy-related genes identified in the previous step were chosen as predictors, and relevant clinical variables such as age, tumor stage, hormone receptor status, and treatment modalities were adjusted for in the analysis.

Also, survival analysis was done to predict survival curves according to autophagy gene expression levels. This was accomplished using the Kaplan-Meier technique, and statistical significance of survival differences between gene expression groups was determined using either log-rank tests or Cox proportional hazards models. Hazard ratios and corresponding confidence intervals were generated to quantify the effect size of autophagy-related gene expression on breast cancer prognosis. We controlled the false discovery rate for both DEG analysis and GSEA by performing multiple testing adjustments using the Benjamini-Hochberg approach. The findings' reliability was further assessed by sensitivity analysis. Finally, the relevance of the discovered genes and pathways in breast cancer prognosis was considered throughout the interpretation and discussion of the findings using data from differential gene expression analysis, GSEA, Cox regression, and survival analysis.

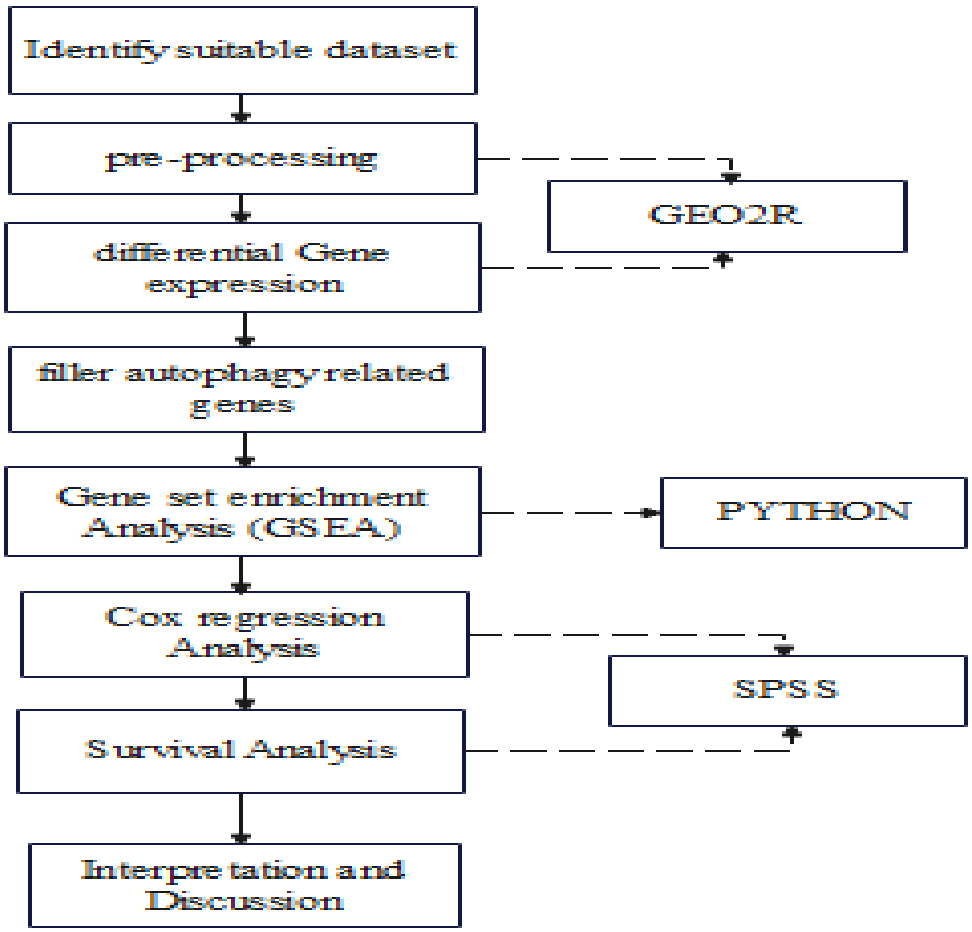


Fig.10 Steps followed in Methodology

6. RESULTS

6.1 Identification of Differentially Expressed Genes:

6.1.1 Data Set and Tool Description

6.1.2 Data Set:

Retrieved from NCBI (https://www.ncbi.nlm.nih.gov/)> GEO DataSets > GSE42568

GSE42568 An analysis of 104 breast cancer biopsies taken from individuals ranging in age from 31 to 89 (mean age = 58) at the time of diagnosis (before tamoxifen or chemotherapy was administered). Seventy-seven women were 50 or older at the time of diagnosis, while twenty were younger. Tumours varied in size from 0.6 to 8.0 centimetres (mean = 2.79 centimetres). T1 tumours had a maximum diameter of less than 2 centimetres; T2 tumours, between 2 and 5 centimetres; and T3 tumours, more than 5 centimetres. There were 82 cases of invasive ductal carcinoma, 17 cases of invasive lobular carcinoma, and 5 cases of rare tumours (2 tubal and 3 mucinous). There were 11 grade 1 tumours, 40 grade 2 tumours, and 53 grade 3 tumours. Oestrogen receptor (ER) positivity was found in 67 tumours, whereas ER negativity was found in 34 (ER status was assessed by Enzyme Immuno-Assay (EIA), with a value of 200 fmol/g protein indicating a positive result). Three patients lacked information on their ER status. Fifty-nine tumours had spread to the axillary lymph nodes, whereas 45 had not. A total of 69 women received post-operative tamoxifen treatment, whereas the remaining 26 did not. Fifty patients were given systemic adjuvant chemotherapy (CMF +/ adriamycin), whereas the other 45 were not. Nine patients lacked complete information on their use of tamoxifen and systemic chemotherapy. The longest period of follow-up was 3,026 days, and the average was 1,887 days. Additionally, 17 control breast tissues were tested.

6.1.2 Tool used (GEO2R)

The National Centre for Biotechnology Information (NCBI) created the GEO2R programme, which is a useful tool for finding differentially expressed genes in gene expression datasets. A number of procedures are necessary to use GEO2R. The user must first browse the GEO2R website and choose their desired dataset by either typing in the GEO Series accession number or by uploading their own. The user then specifies the experimental parameters or study groups to

compare throughout the dataset. The required statistical approach (for example, t-test, linear regression, or ANOVA) and multiple testing adjustments are given as statistical analysis parameters. GEO2R starts the analysis, does the statistical calculations, and creates a table of the results. The statistics at the gene level, including p-values, fold changes, and corrected p-values, are detailed in this table. The conventional method for finding differentially expressed genes is to use statistical significance and fold change thresholds. The data can then be interpreted by researchers, who will concentrate on the identified genes and the biological processes and pathways they are connected with. By utilising GEO2R, researchers are given an easy-to-use and effective tool for identifying differentially expressed genes. This aids in the understanding of gene expression patterns and potential biological mechanisms underlying a specific condition or experimental treatment.

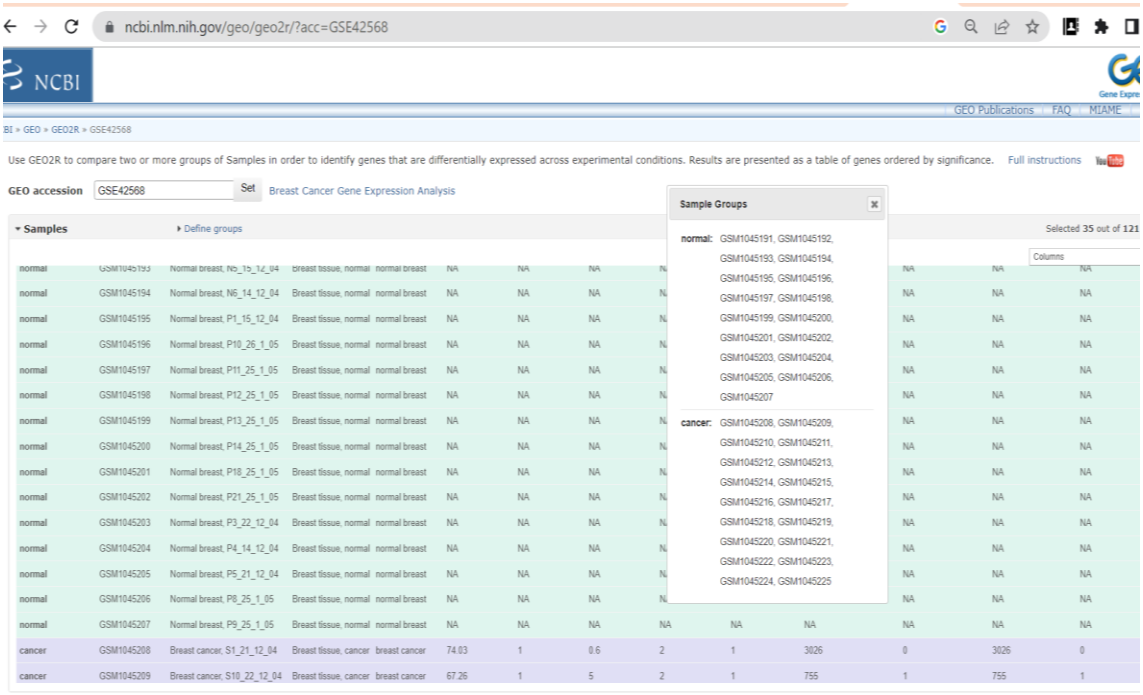


Fig.11 GSE42568 data set in GEO2R tool

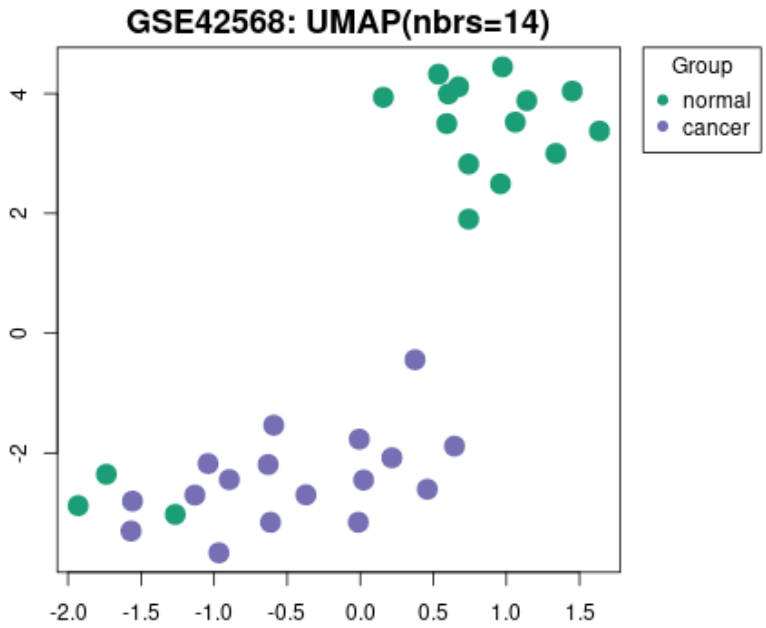


Fig.12 UMAP(Uniform Manifold Approximation and Projection) Plot between normal & cancer samples

Figure 12 presents a UMAP (Uniform Manifold Approximation and Projection) plot that visualizes the differential gene expression patterns between normal and cancer samples.The coordinates or positions of the data points in the reduced-dimensional space are commonly represented by the X and Y labels in the context of dimensionality reduction techniques like UMAP (Uniform Manifold Approximation and Projection).The UMAP algorithm is a dimensionality reduction method for representing high-dimensional data in a lower-dimensional space while maintaining the original data's structure and connections. The plot consists of a two-dimensional representation of the samples, where each point represents an individual sample. The samples are color-coded based on their classification as either normal or cancer. By applying differential expression analysis (DEA) techniques, genes that show significant differences in expression between normal and cancer samples were identified. The UMAP plot overlays these differentially expressed genes onto the samples, allowing for a visual exploration of the gene expression patterns associated with normal and cancer conditions. The color intensity or shading of the points on the UMAP plot may reflect the expression levels of specific genes or a composite score representing the overall gene expression pattern. Genes with higher expression

levels in cancer samples may be represented by darker or more intense colors, while genes with higher expression levels in normal samples may be represented by lighter or less intense colors. The UMAP plot facilitates the identification of clusters or distinct groups of samples based on their gene expression profiles. It provides insights into the underlying molecular heterogeneity between normal and cancer samples and allows for the identification of genes that contribute to the separation between these groups. The UMAP plot is a valuable tool for visualizing and exploring the differences in gene expression patterns between normal and cancer samples. It helps find genes of interest or biomarkers that may be important in the onset or course of illness.

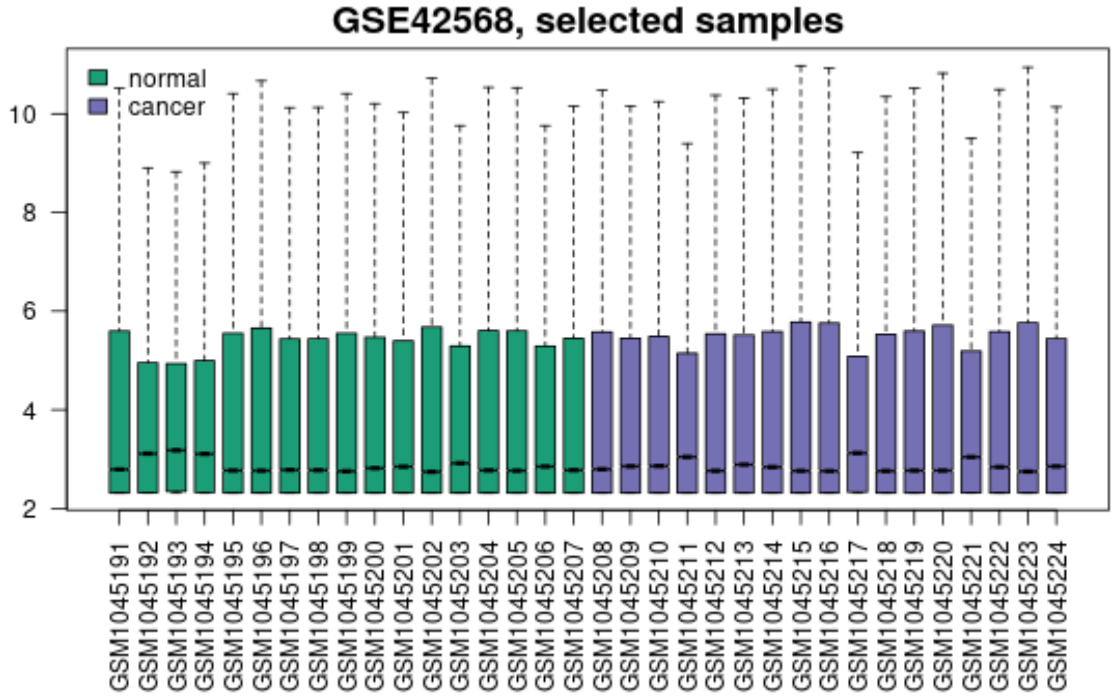


Fig.13 Selected Samples out of the GSE42568

Gene expression profiles **(GSM---)** for a few representative average as well as cancer samples are shown side by side in Figure 2. This illustration is meant to draw attention to the contrast between the two groups concerning gene expression patterns. The genes or characteristics under investigation are shown along the x-axis, while expression levels are shown along the y-axis. The expression levels of each gene in both the normal and cancer samples are shown graphically by individual points and lines. Variations in gene expression between normal and malignant samples may be visually evaluated using the plot. Variations in gene expression may provide

light on the molecular alterations that underlie cancer initiation and progression. The plot may show distinct patterns, such as upregulation or downregulation of certain genes in the cancer samples compared to the normal samples. These differential expression patterns may help identify key genes or pathways involved in the development or progression of cancer.

Additionally, the plot may reveal clusters or groups of genes that exhibit coordinated expression changes in the cancer samples compared to the normal samples. Such clusters may represent gene sets or pathways that are collectively dysregulated in cancer.Researchers can use this plot to generate hypotheses about the molecular mechanisms underlying the observed differences in gene expression between normal and cancer samples. Further analysis and functional interpretation of the identified differentially expressed genes can provide valuable insights into the biological processes associated with cancer development and progression.

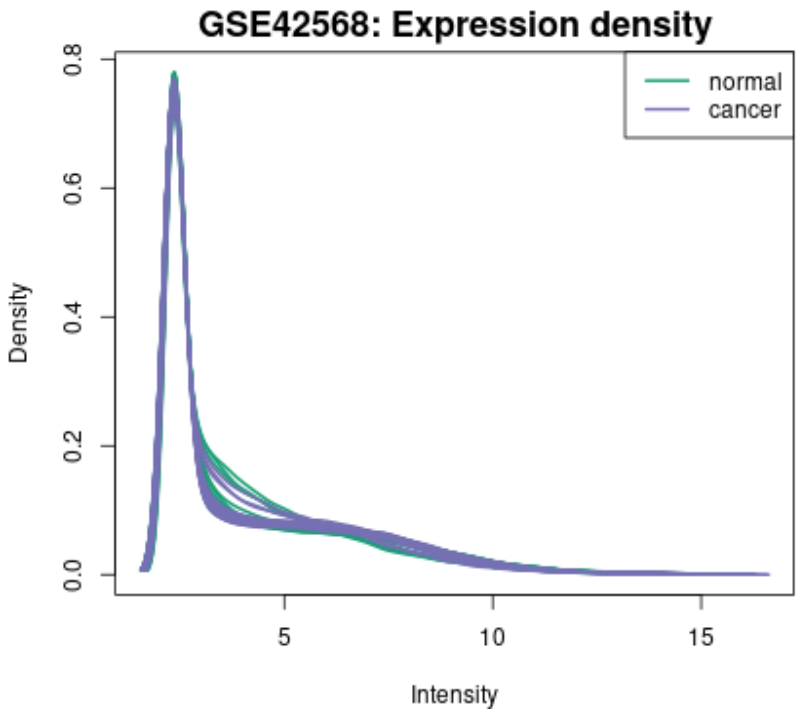


Fig.14 Intensity vs Density of gene expression levels

Figure 14 presents a density vs intensity plot that provides insights into the distribution and intensity of gene expression levels in the studied samples. This plot aids in understanding the overall expression patterns and variability of genes of interest.The y-axis shows the density of gene expression values, while the x-axis shows the intensity or degree of gene expression. The

density curve shows the distribution of gene expression levels across the samples, indicating the frequency of occurrence for different intensity values.The density vs intensity plot allows for the identification of key features in the gene expression data, such as the presence of distinct expression groups or the presence of outliers. It helps assess the spread and central tendency of gene expression values, providing valuable information about the overall gene expression landscape.The plot can reveal whether the gene expression values follow a normal distribution or exhibit skewness or multimodality. Skewed distributions may indicate asymmetry or bias in gene expression, while multimodal distributions may suggest the presence of distinct subgroups or expression patterns within the samples.

The density versus intensity map may also be used to evaluate the variation in gene expression across groups. By overlaying multiple density curves on the same plot, it becomes possible to visually compare the distribution and intensity of gene expression across various conditions or experimental factors.The density vs intensity plot serves as a visual summary of the gene expression data, providing an overview of the distribution and intensity of expression levels. It helps researchers identify patterns, outliers, and potential trends within the dataset, leading to further exploration and analysis of gene expression patterns.

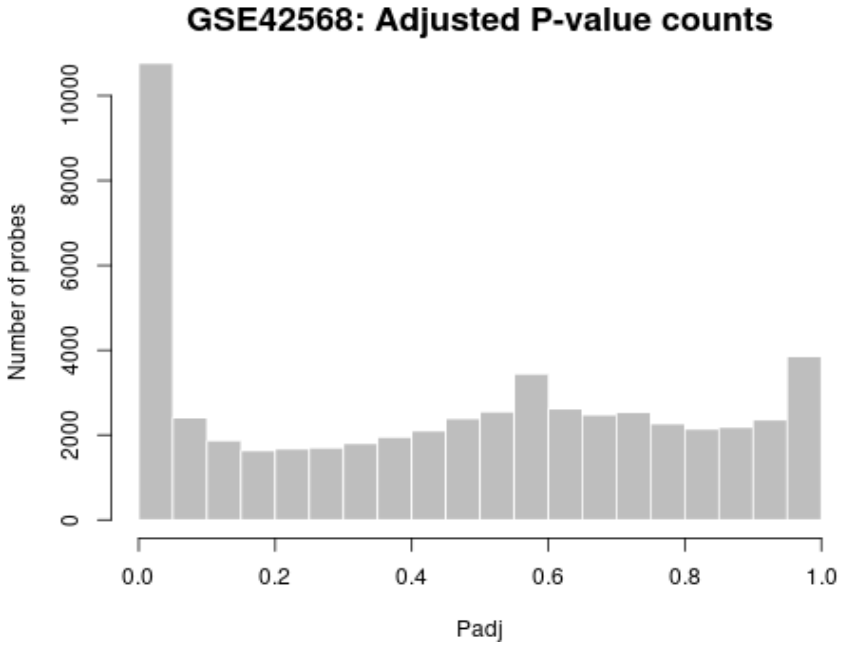


Fig.15 Adjusted p-value counts

Figure 15 presents an adjusted p-value counts plot that provides insights into the statistical significance of differential gene expression analysis. Differing settings or sample groups may be compared using this plot to locate genes with significantly different expression levels. Adjusted p-values, computed to account for multiple testing and regulate the false discovery rate (FDR), are shown on the x-axis. The number of genes that fall within a specific adjusted p-value range is shown along the y-axis. The adjusted p-value counts plot allows for the identification of significantly differentially expressed genes by observing the distribution of counts across different levels of statistical significance. Genes with lower adjusted p-values are considered more statistically significant and are often associated with stronger evidence for differential expression.The plot typically exhibits a peak or spike at lower adjusted p-values, indicating a larger number of genes that show significant changes in expression levels. As the adjusted p-values increase, the count of genes decreases, indicating fewer genes with less significant changes in expression.

This plot aids in determining the appropriate threshold for identifying differentially expressed genes based on the desired level of statistical significance. Researchers can select a specific adjusted p-value cut-off based on their study objectives and the significance level they deem appropriate.The adjusted p-value counts plot provides a visual representation of the results of differential gene expression analysis, highlighting the genes with the most significant changes in expression levels. It helps researchers identify potential candidate genes for further investigation and exploration of biological processes or pathways associated with the studied conditions.

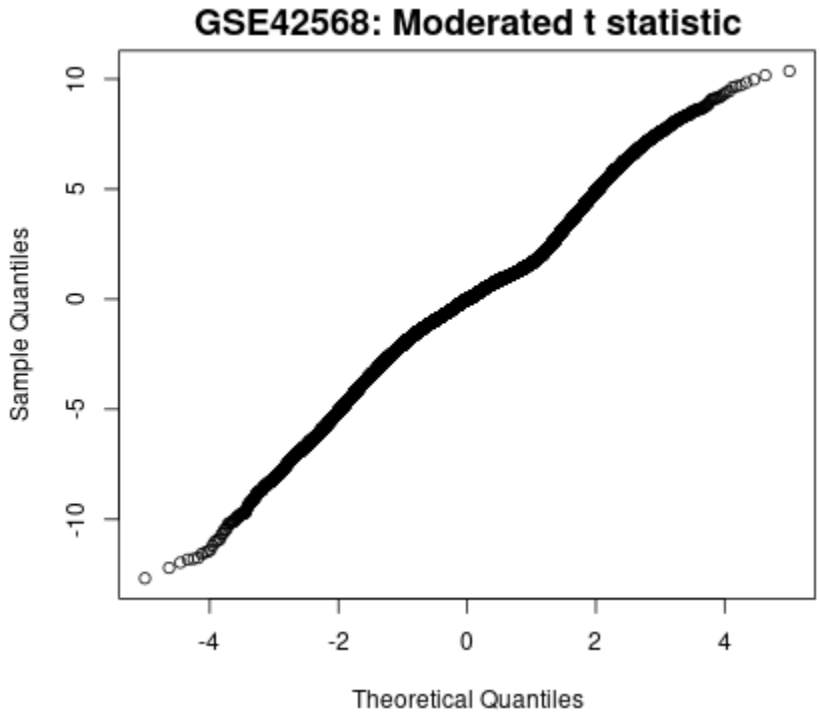


Fig.16 Moderated t statistics

Figure 16 presents a theoretical quantities vs sample quantities plot, which is a graphical tool used to assess the similarity or dissimilarity between the theoretical distribution and the observed sample distribution. This plot aids in evaluating how well the data fits a specific theoretical distribution or model.The x-axis represents the theoretical quantities or values expected under a specific distribution, while the y-axis represents the corresponding sample quantities or values observed in the actual dataset. Each data point on the plot represents a specific quantile or value from the sample.The plot allows for a visual comparison between the theoretical distribution and the sample distribution. If the sample data closely follows the theoretical quantities, the plot will show a linear relationship with the points lying along a straight line. On the other hand, deviations from the theoretical line suggest discrepancies or differences between the observed data and the expected distribution.By comparing the plot to the ideal straight line, researchers can evaluate the goodness-of-fit of the data to the theoretical distribution. If the points align closely with the line, it suggests that the data fits the theoretical distribution well. Conversely, if the points deviate significantly from the line, it indicates a poor fit or potential deviation from the assumed distribution.

The theoretical quantities vs sample quantities plot provides insights into the appropriateness of the chosen distribution or model for the dataset. It helps researchers assess whether the assumed distribution accurately represents the observed data or if alternative distributional assumptions may be more appropriate.Additionally, this plot aids in identifying potential outliers or data points that deviate significantly from the expected distribution. Such outliers may indicate unusual observations or data quality issues that require further investigation.

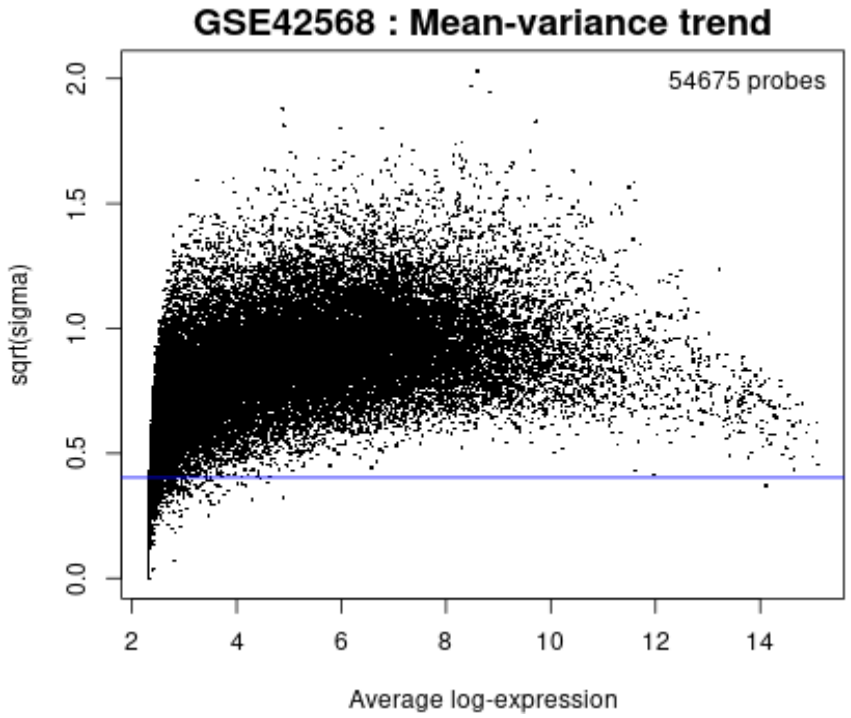


Fig.17 Mean-Variance Trend

Fig.17 presents an average log expression vs square root of sigma (σ) plot, which is a graphical tool used to assess the relationship between the average gene expression and the variability of gene expression across samples.The x-axis represents the average log expression, which is calculated as the average of the logarithm-transformed expression values for each gene across all samples. The y-axis represents the square root of sigma (σ), where sigma represents the standard deviation or variability of the gene expression values.The scatter plot illustrates the connection between average gene expression and sample variation. It provides insights into how the gene's expression varies across different conditions or experimental factors.In the plot, each data point represents a specific gene, and its position reflects the average log expression value on the x-axis

as well as the square root of sigma on the y-axis. By analysing the distribution of data points, researchers can identify patterns or trends in gene expression variability relative to the average expression level.Typically, genes with higher average log expression values and lower square root of sigma values are considered more stable or consistently expressed across samples. These genes exhibit lower variability and are less likely to be influenced by experimental noise or technical artifacts.

Conversely, genes with lower average log expression values and higher square root of sigma values indicate higher variability in their expression levels across samples. These genes may be influenced by various biological or environmental factors, leading to greater fluctuations in their expression patterns.The average log expression vs square root of sigma plot helps researchers identify genes with stable expression patterns and those that are more variable or context-dependent. This information can guide further investigations into the biological processes and regulatory mechanisms associated with differentially expressed genes.

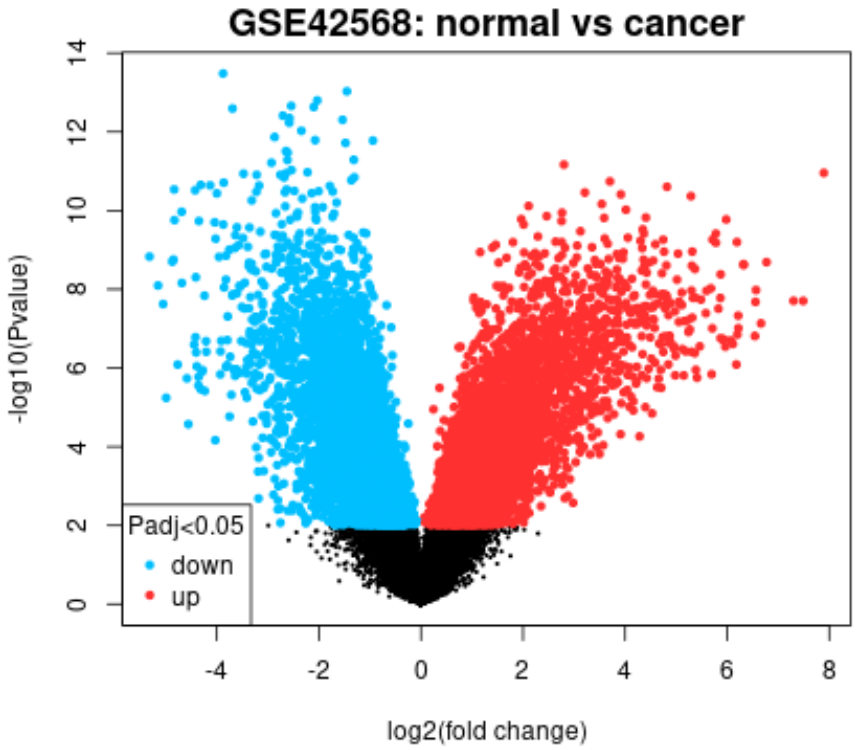


Fig.18 log2(fold change) vs log10 (p-value)

The findings of the differential gene expression study are graphically represented in Figure 18 as a volcano plot, where the log2(fold change) is shown on the x-axis as well as the negative logarithm (base 10) of the p-value is plotted on the y-axis. The x-axis shows the log2(fold change), which measures the degree to which gene expression differs between two conditions or groups. Upregulation is represented by positive numbers and downregulation by negative ones. The p-value, which shows the importance of the observed fold change, is shown as the negative logarithm (base 10) on the y-axis. A greater degree of differential expression corresponds to a higher value. Each data point in the volcano plot corresponds to a single gene and is located on the x-axis according to its log2(fold change) and on the y-axis according to its negative log10(p-value). The degree of change as well as the statistical significance of variations in gene expression, may be shown in the same graph. The volcano plot aids in the identification of genes that exhibit statistically significant changes in expression levels. Genes located toward the top of the plot with high negative log10(p-values) are considered highly significant, indicating a strong association between the observed fold change and the likelihood of differential expression.

Furthermore, the volcano plot allows for the identification of genes with large fold changes, regardless of statistical significance. Genes located on the far left or right sides of the plot with high absolute values of log2(fold change) indicate substantial changes in expression levels, regardless of whether the changes are statistically significant.The plot also helps in identifying differentially expressed genes that fall within a specific range of fold change and statistical significance. Genes that fall within the central region of the plot may exhibit moderate changes in expression levels with moderate statistical significance.Researchers can use the volcano plot to prioritize genes for further investigation based on their position in the plot. Genes located in the upper-right or upper-left corners of the plot, representing significant fold changes with high statistical significance, are often considered as strong candidate genes for further analysis.

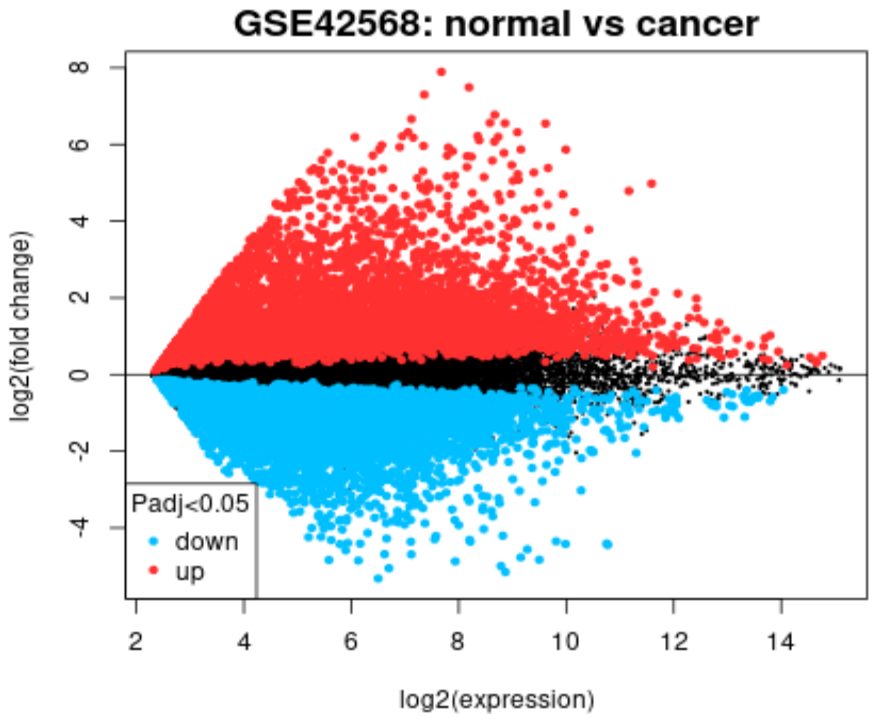


Fig.19 log2(expression) vs log2(fold change)

The findings of the differential gene expression study are graphically shown in Figure 19 as a volcano plot, where log2(expression) is plotted on the x-axis and log10(fold change) is plotted on the y-axis. Gene expression is shown on a logarithmic scale, denoted by the x-axis variable log2(expression). Higher numbers suggest more expressiveness, whereas lower values indicate less. Changes in gene expression between two conditions or groups are shown by the log10(fold change) on the y-axis. Upregulation is represented by positive numbers and downregulation by negative ones. Each point in the volcano plot corresponds to a single gene, and its location on the axes indicates the log2(expression) and log10(fold change) of that gene, respectively. The plot allows for the simultaneous visualization of both the expression level and the magnitude of change in gene expression.The volcano plot aids in the identification of genes that exhibit significant changes in expression levels. Genes located toward the top of the plot with high positive or negative log10(fold change) values indicate substantial changes in expression, regardless of the actual expression level. These genes are considered as potential candidates for differential expression analysis.

Furthermore, the volcano plot allows for the identification of genes with different expression levels. Genes located toward the left or right sides of the plot with high positive or negative

log2(expression) values indicate high or low expression levels, respectively.Researchers can use the volcano plot to prioritize genes for further investigation based on their position in the plot. Genes located in the upper quadrants of the plot, representing significant fold changes with high expression levels or low expression levels, are often considered as strong candidates for further analysis.It is important to note that the significance of differential gene expression should be assessed in conjunction with statistical tests, such as p-values or adjusted p-values, to determine the statistical significance of observed changes.

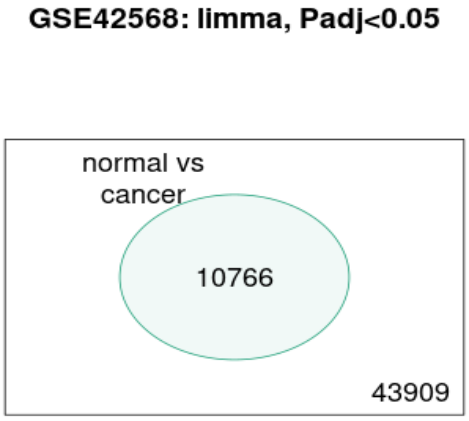


Fig.20 Venn Diagram for finding out Autophagy Related Differentially Expressed Genes

6.2 **GSEA** (Gene Set Enrichment Analysis)

A computational technique called **Gene Set Enrichment Analysis** (GSEA) **or Functional Enrichment Analysis** or **Pathway Enrichment Analysis** examines whether a set of genes that has been predetermined exhibits statistically significant, concordant differences between two biological states (such as phenotypes).

The method's effectiveness comes from a focus on gene sets, or collections of genes with similar biological functions, chromosomal locations, or regulatory mechanisms. Notably, GSEA identifies numerous biochemical pathways in common, whereas single-gene analysis found little overlap between two independent studies of patient survival in breast cancer. We used the required packages in Python for obtaining the enrichment plots for different GO (Gene Ontology).

The following is the Python Code used for performing Gene Set Enrichment Analysis:

```
!pip install gseapy
import pandas as pd
fromgseapy.plotimportgseaplot
importgseapyasgp
importnumpyas np
df = pd.read_table('GSE42568_GSEA.tsv').dropna()
df = df.rename(columns = {'symbol': 'Gene'})
df['Rank'] = -np.log10(df['adj.P.Val'])*df.logFC

df = df.sort_values('Rank', ascending = False).reset_index(drop = True)
ranking = df[['Gene.symbol', 'Rank']]
ranking[ranking['Gene.symbol']=='ATG16L1']
gp.get_library_name()
pre_res = gp.prerank(rnk = ranking, gene_sets = 'GO_Biological_Process_2021', seed = 6,
permutation_num = 100)
pre_res.results[term]
out = []

for term inlist(pre_res.results):
   out.append([term,
            pre_res.results[term]['fdr'],
            pre_res.results[term]['es'],
            pre_res.results[term]['nes'], pre_res.results[term]['lead_genes']])

out_df = pd.DataFrame(out, columns = ['Term','fdr', 'es',
'nes','lead_genes']).sort_values('fdr').reset_index(drop = True)
out_df
term_to_graph = out_df.iloc[1004].Term
term_to_graph
gseaplot(pre_res.ranking, term = term_to_graph, **pre_res.results[term_to_graph])
```

6.2.1 Autophagy of mitochondrion (GO:0000422)

The autophagy genes ATG12, ATG3, ATG14, ATG5, ULK2, BNIP3, BECN1 emerged as lead genes associated with the 'cellular protein localization' gene ontology term (GO:0034613). The enrichment analysis revealed a notable statistical significance with an FDR value of0.443811, suggesting a robust association. The expression levels of these autophagy genes are positively correlated with the process of cellular protein localisation, as shown by the ES of 0.778362. This

suggests that these genes serve crucial functions in ensuring that proteins are correctly localised inside cells. Furthermore, the Normalized Enrichment Score (NES) of 1.252036underscores the reliability and strength of the enrichment signal, emphasizing the significance of these autophagy genes in driving the observed enrichment within the gene ontology category.

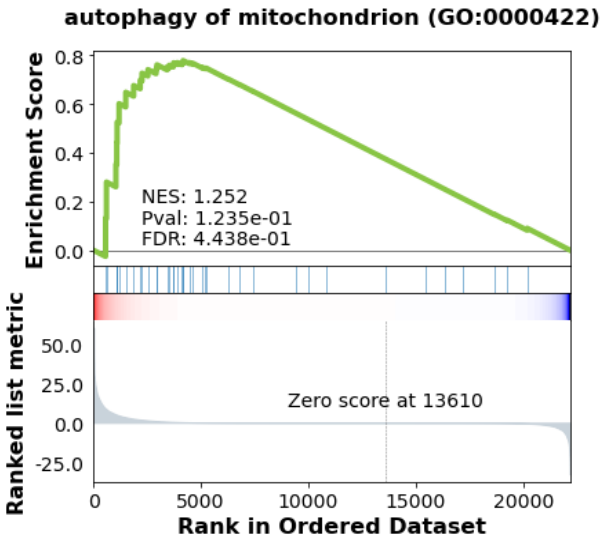


Fig. 21 Enrichment score of autophagy of mitochondrion

6.2.2 Mitochondrion Disassembly (GO:0061726)

The autophagy genes ATG12, ATG3, BECN1, and ATG5 were identified as lead genes associated with the 'mitochondrion disassembly' gene ontology term (GO:0061726). The enrichment analysis revealed an FDR value of 0.516567877, indicating a moderate level of statistical significance. With an Enrichment Score (ES) of 0.7804762931, this set of autophagy genes seems to have a favourable relationship with mitochondrial disintegration. This suggests that these genes are involved in the essential process of mitochondrial disassembly, which maintains cellular balance. Furthermore, the Normalized Enrichment Score (NES) of 1.222673018 highlights the reliability and strength of the enrichment signal, emphasizing the significance of these autophagy genes in driving the observed enrichment within the gene ontology category.

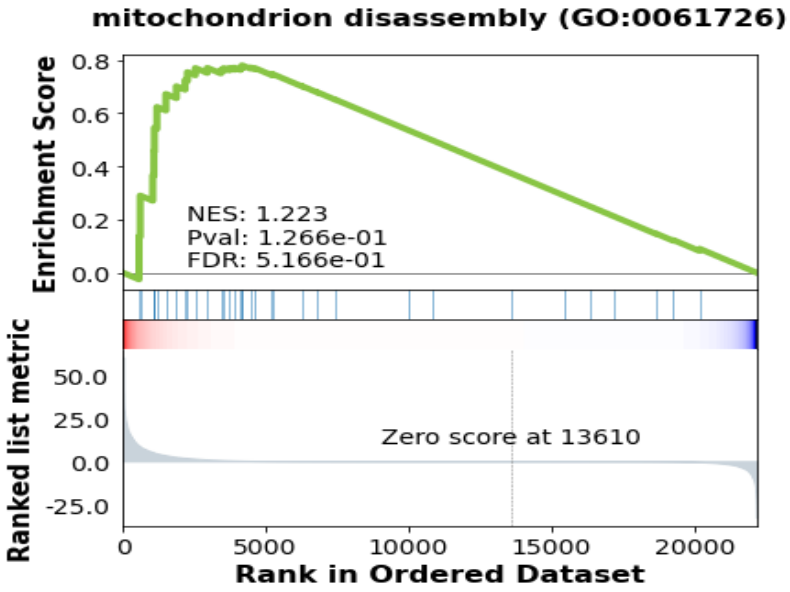


Fig.22 Enrichment score of mitochondrion disassembly

6.2.3 Autophagosome Maturation (GO:0097352)

The autophagy genes ATG14 and LAMP2 were identified as lead genes associated with the autophagosome maturation (GO:0097352)The enrichment analysis revealed an FDR value of 0.557887, indicating a moderate level of statistical significance. There seems to be a positive relationship between the expression of these autophagy genes as well as the disintegration of mitochondria, as shown by the ES of 0.751608. This suggests that these genes are involved in the essential process of mitochondrial disassembly, which maintains cellular balance. Furthermore, the Normalized Enrichment Score (NES) of 1.2078highlights the reliability and strength of the enrichment signal, emphasizing the significance of these autophagy genes in driving the observed enrichment within the gene ontology category.

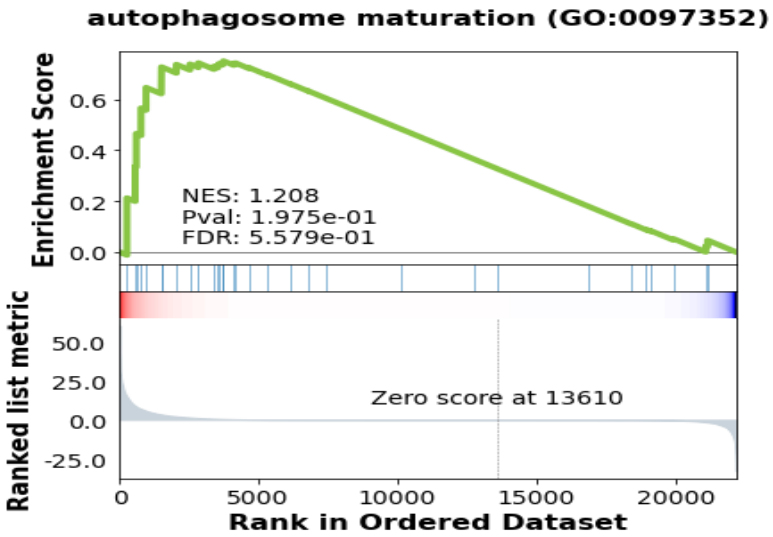


Fig.23 Enrichment score of autophagosome maturation

6.2.4 Selective Autophagy (GO:0061912)

The autophagy genes ATG14,BECN1, and ULK2 were identified as lead genes associated with the selective autophagy (GO:0061912) The enrichment analysis revealed an FDR value of 0.565275, indicating a moderate level of statistical significance. There may be a connection between the expression of these autophagy genes as well as the breakdown of mitochondria, as shown by the ES of 0.709114. This suggests that these genes are involved in the essential process of mitochondrial disassembly, which maintains cellular balance. Furthermore, the Normalized Enrichment Score (NES) of 1.203096highlights the reliability and strength of the enrichment signal, emphasizing the significance of these autophagy genes in driving the observed enrichment within the gene ontology category.

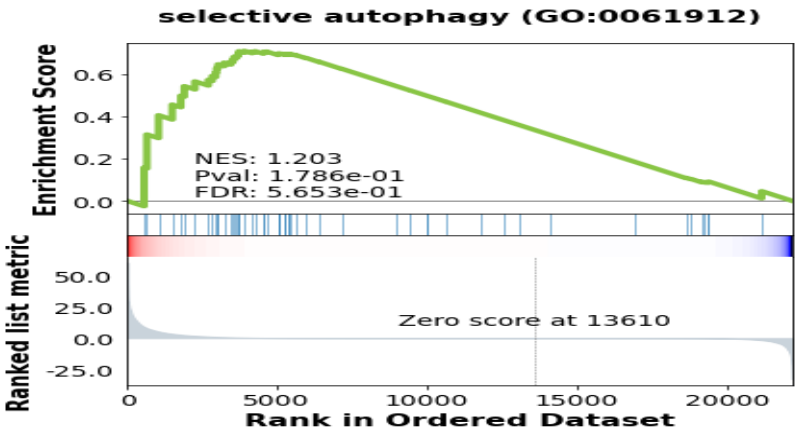


Fig.24 Enrichment score of selective autophagy

6.2.5 Aggrephagy (GO:0035973)

The autophagy genes ATG5, and SQSTM1 were identified as lead genes associated with the aggrephagy (GO:0035973)The enrichment analysis revealed an FDR value of 0.636682,indicating a moderate level of statistical significance. The expression levels of these autophagy genes seem to be positively correlated with mitochondrial disintegration, as shown by the ES of 0.761659. This suggests that these genes are involved in the essential process of mitochondrial disassembly, which maintains cellular balance. Furthermore, the Normalized Enrichment Score (NES) of 1.162665highlights the reliability and strength of the enrichment signal, emphasizing the significance of these autophagy genes in driving the observed enrichment within the gene ontology category.

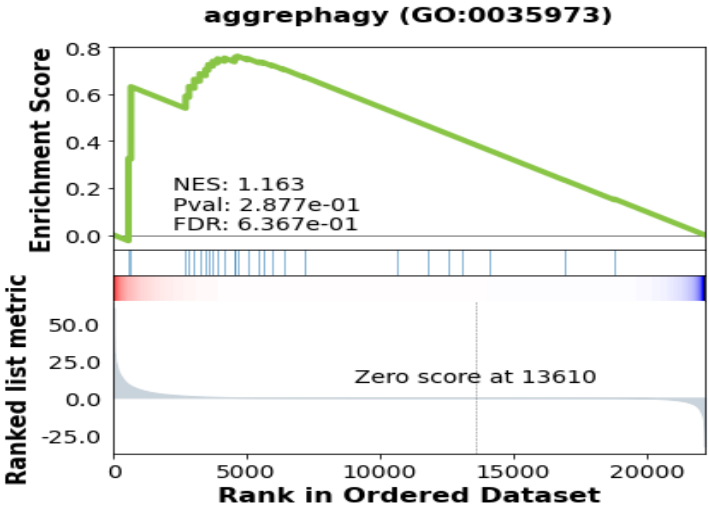


Fig.25 Enrichment score of aggrephagy

6.3 Survival Analysis

6.3.1Cox Regression

Table 3 Table of Coefficients

Gene ID	Cox Coefficient	P-Value	FDR Corrected	Rank	Median Expression	Mean Expression	log-Rank-p
ATG12	0.245	6.10E-03	2.08E-01	480	543.55	572.8	0

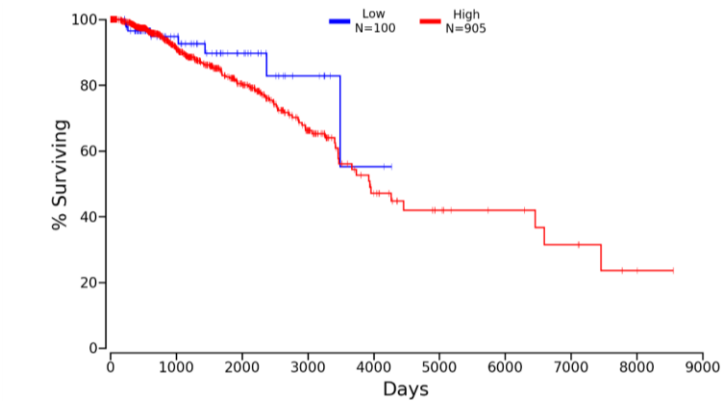
ATG3	0.065	4.90E-01	7.94E-01	10142	822	870.93	0.1
ATG14	-0.134	1.60E-01	5.32E-01	4895	543.95	553.53	0.1
ATG5	0.202	3.20E-02	3.23E-01	1615	763.48	904.67	0.5
BECN1	-0.077	3.80E-01	7.22E-01	8606	1762.99	1862.58	0.2
ULK2	-0.105	2.40E-01	6.17E-01	6457	616.4	743.84	0.2
BNIP3	0.122	2.00E-01	5.79E-01	5594	809.71	1023.92	0.9
LAMP2	0.142	9.70E-02	4.50E-01	3507	5118.73	5680.26	0.02
SQSTM1	0.016	8.50E-01	9.47E-01	14919	9003.17	10475.89	0.5

Table 3 shows the findings of a Cox regression research into the correlation between autophagy gene expression and breast cancer patient survival rates. Nine autophagy-related genes (ATG12, ATG3, ATG14, ATG5, BECN1, ULK2, BNIP3, LAMP2, as well as SQSTM1) have their Cox coefficients, p-values, FDR-corrected p-values, ranks, median expression, mean expression, and log-rank p-values listed in the table. The Cox coefficient represents the estimated coefficient for each autophagy gene in the Cox regression model, indicating the direction and magnitude of its impact on survival. The significance of the link between gene expression and survival is represented by the p-value. The FDR corrected p-value is a more conservative measure that takes into account multiple testing and adjusts the significance threshold accordingly.The rank column provides the ranking of each gene based on its association with survival outcomes. A lower rank indicates a stronger association. Median expression and mean expression values represent the median and mean expression levels of the gene in the studied cohort, giving information on the autophagy gene expression patterns in breast cancer. Final statistical significance of survival differences between groups of autophagy gene expression is evaluated by the log-rank p-value. A significant log-rank p-value suggests that the gene's expression levels have a significant impact on patient survival. The data in the table add to our knowledge of autophagy-related genes and their impact on breast cancer prognosis. Taking into account the Cox coefficient, p-value, p-value after false discovery rate adjustment, rank, expression levels, as well as log-rank p-values

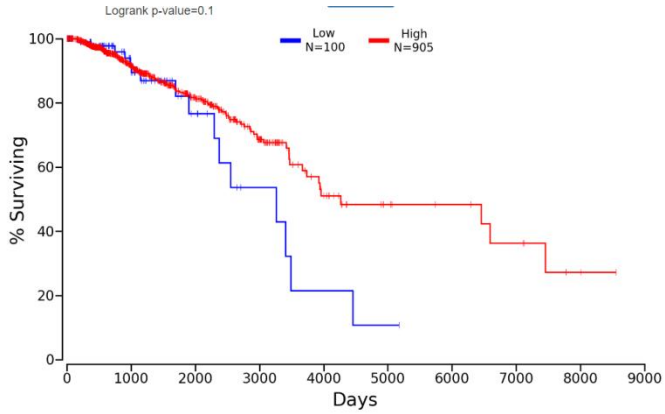
for the autophagy genes investigated, further interpretation and discussion is based on the relevance of the discovered genes and pathways in breast cancer prognosis**.**

6.3.2 Kaplan-Meier Survival Plots

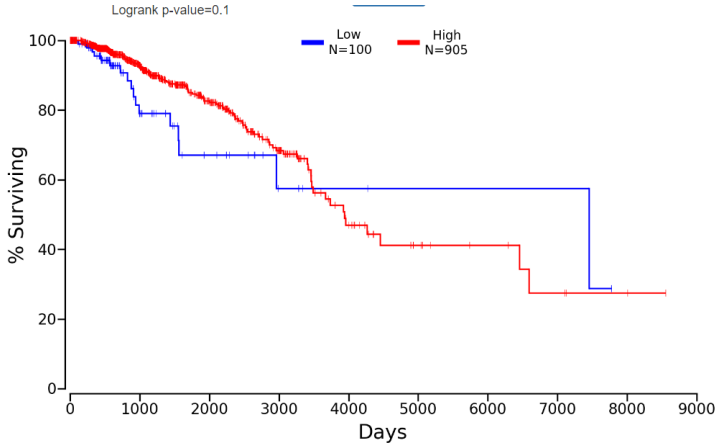
ATG12:



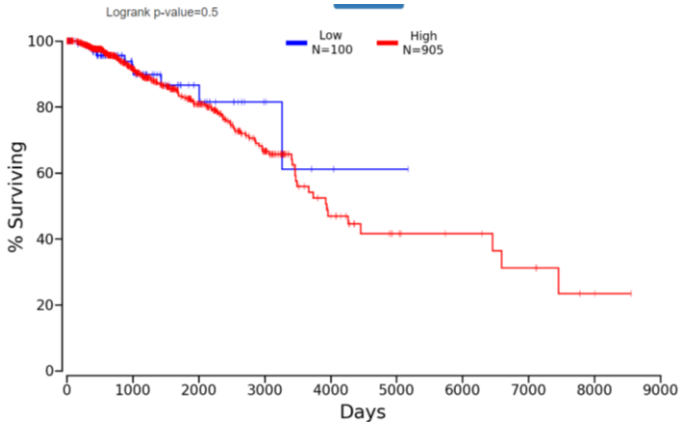
ATG3



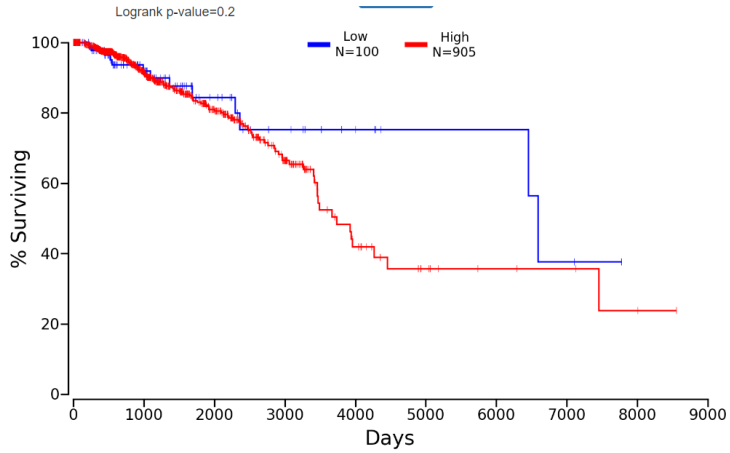
ATG14



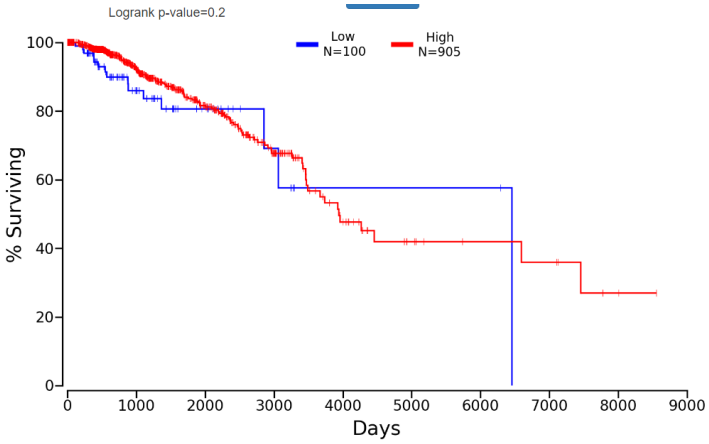
ATG5



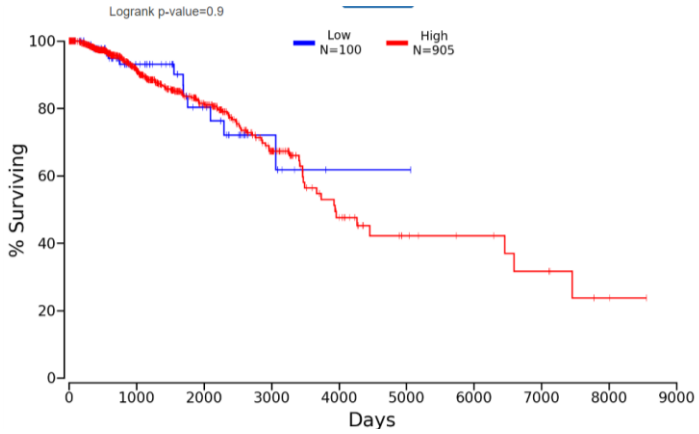
BECN1



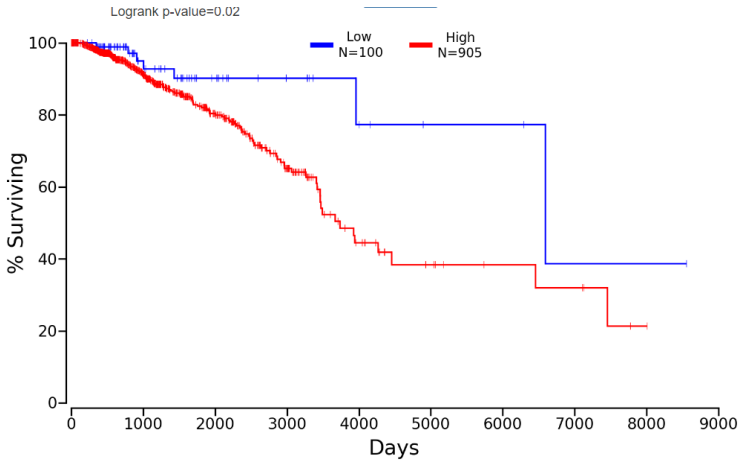
ULK2



BNIP3



LAMP2



SQSTM1

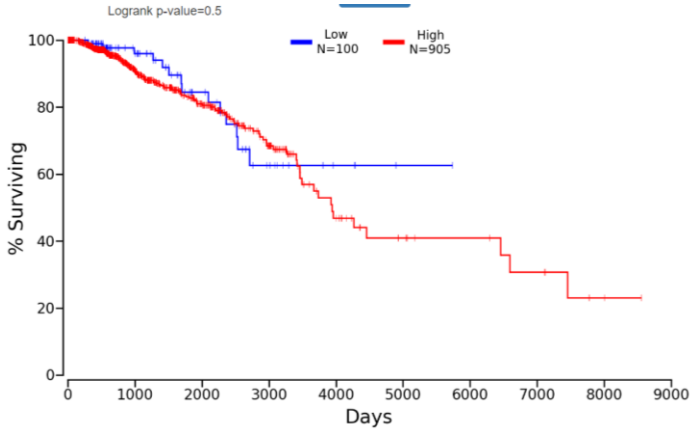


Fig.26.Kaplan-Meier Survival Plots of 9 Autophagy Related Genes

Figure 26 presents the Kaplan-Meier survival plot illustrating the impact of autophagy-related genes on the survival outcomes of breast cancer patients. Plotted using the web app

(http://www.oncolnc.org/search_results/?q=brust) The scatter plot reveals important information about the correlation between the expression of these genes and the likelihood of a patient's survival. Months of time on the x-axis, and the likelihood of survival on the y-axis. The plot includes separate curves for each autophagy-related gene, namely ATG12, ATG3, ATG14, ATG5, BECN1, ULK2, BNIP3, LAMP2, and SQSTM1. Each curve depicts the survival probability over time based on the gene's expression level. varying sets of autophagy-related gene expression show varying survival probability, as shown by the survival curves. Distinct patterns of survival are linked to different degrees of gene expression, as shown by the space between the curves. To determine whether or not there were statistically significant variations in survival rates between the groups, researchers utilised either the log-rank test or the Cox proportional hazards model. The hazard ratio (HR) and corresponding confidence intervals (CIs) associated with each gene's expression levels can be calculated from the Cox regression analysis. The HR quantifies the relative risk of an event (such as death or disease progression) between different expression groups of the gene. A HR greater than 1 indicates a higher risk or poorer prognosis, while a HR less than 1 suggests a lower risk or better prognosis. The Kaplan-Meier survival plot serves as a visual representation of the impact of autophagy-related genes on breast cancer prognosis. It provides valuable insights into the potential prognostic significance of these genes and helps identify subgroups of patients with distinct survival outcomes based on their gene expression patterns

Table 4 Summary effect of autophagy genes on the prognosis of Breast Cancer:

Random-Effects Model (k = 9)						
	Estimate	**se**	**Z**	**p**	**CI Lower Bound**	**CI Upper Bound**
Intercept	0.0538	0.0462	1.16	0.244	-0.037	0.144
Note. Tau² Estimator: Restricted Maximum-Likelihood						

Heterogeneity Statistics							
Tau	**Tau²**	**I²**	**H²**	**R²**	**df**	**Q**	**p**

0.135	0.0183 (SE= 0.0096)	95.20%	20.837	.	8	168.094	< .001

The Fisher r-to-z transformed Cox regression was used for the study. The data were analyzed using a random-effects model. We used the constrained maximum-likelihood estimator (Viechtbauer 2005) to assess the degree of heterogeneity (tau^2). The Q-test for heterogeneity (Cochran 1954) as well as the I^2 statistic, are provided with the estimate of tau2. A prediction range for the actual outcomes is also supplied in case any heterogeneity is found (i.e., $tau^2 > 0$ regardless of the findings of the Q-test). We employ studentized residuals and cook's distances to determine whether the autophagy gene is an outlier and/or essential in the model. Using a Bonferroni correction with two-sided alpha = 0.05 for k Autophagy genes included in the meta-analysis, genes with a studentized residual greater than the 100 x (1 - 0.05/(2 X k))th percentile of a standard normal distribution are considered outliers. To be deemed important, an autophagy gene has to have a Cook distance that is more than the median plus six times the interquartile range of Cook's distances. To examine if a funnel plot is skewed, researchers may use the rank correlation test or a regression test using the standard error of the observed results as the predictor.

There were a total of k=9 Autophagy genes considered. Most estimates (67%) were on the positive side of the spectrum for the Fisher r-to-z transformed cox regression. Using a random-effects model, we calculated that the average Fisher r-to-z transformed cox regression was \hat{\mu = 0.0538 (95% CI: -0.0368 to 0.1444). Consequently, the mean result did not deviate noticeably from zero (z = 1.1646, p = 0.2442). The Q-test indicates that the actual results are not normally distributed (Q(8) = 168.0942, p 0.0001, $tau^2 = 0.0183$, $I^2 = 95.2009\%$). The range of -0.2264 to 0.3340 is a 95% confidence interval for the actual results. Therefore, although the average result is assumed to be good, the actual outcome may be negative in certain instances. Studentized residuals were examined, and it was found that no study had a value greater than 2.7729, indicating that there were no outliers for this model. Using Cook's distances, it's clear that all of the investigations have little weight. No funnel plot asymmetry was found using either the rank correlation or the regression test (p = 0.6985 as well as p = 0.7860, respectively).

6.3.3 **Forest Plot**

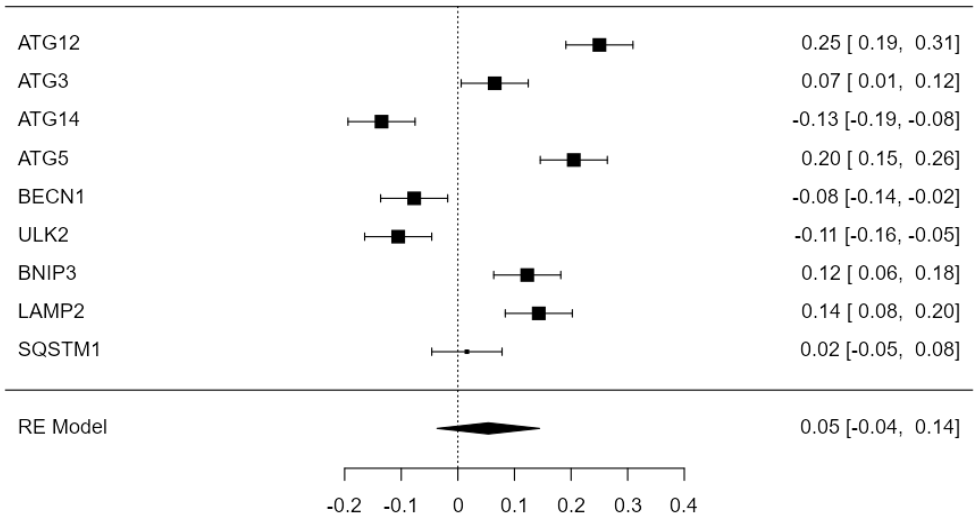


Fig.27 Forest Plot of the 9 Autophagy related genes

7. DISCUSSION

The survival prognosis of breast cancer patients has significantly improved in clinical practise, but metastasis and recurrence rates, which were the main causes of breast cancer death, still need to be reduced. Large-scale investigations have shown that autophagy has a dual role in the emergence of cancer. Accordingly, autophagy can have either a pro-survival or pro-death function in breast cancer depending on the situation . By increasing the survival time of latent breast cancer cells, autophagy encourages metastatic breast cancer recurrence . Triple-negative breast cancer cells' aggressiveness was suppressed by cytostatic autophagy. Additionally, a growing body of research suggests autophagy-related genes have critical roles in the development or prevention of a variety of malignancies, including breast cancer, hepatocellular carcinoma, and lung cancer . finding potential particular ARGs is thus necessary.

In our model , the coefficients in Cox regression survival model are related to hazardous conditions for eg. in our case model the autophagy genes **(ATG12, ATG3,ATG5,BNIP3,LAMP2,SQSTM1)** showing posititive Cox coefficient signifies a worse

prognosis while genes with negative coefficient **(ATG14, BECN1,ULK2)** indicates good prognosis in case of Breast Cancer. Thus, attention was drawn to the possibility of finding specific ARGs having prognostic relevance. The risk model of the nine Autophagy Related Genes ARGs was recognised in this investigation as a standalone predictive factor for breast cancer.

7. CONCLUSION

In conclusion, our study aimed to investigate the role of autophagy-related genes in the prognosis of breast cancer using a comprehensive methodology involving the analysis of public gene expression datasets. Through differential gene expression analysis, we identified a list of differentially expressed genes (DEGs) between breast cancer samples and normal tissue controls. By filtering this DEG list, we focused specifically on autophagy-related genes, which are known to play a crucial role in cellular processes. Gene set enrichment analysis (GSEA) allowed us to assess the enrichment of autophagy-related gene sets within the identified DEGs. We observed significant enrichment, indicating the involvement of autophagy pathways in breast cancer. Cox regression analysis was then performed to evaluate the association between the expression levels of autophagy-related genes***(ATG3,ATG4,ATG5,ATG12,ATG14,ULK2,BCN1,BNIP3,SQSTM1)*** and survival outcomes in breast cancer patients. By adjusting for relevant clinical variables, such as age, tumour stage, hormone receptor status, and treatment modalities, we aimed to identify the independent prognostic significance of autophagy-related genes. Survival analysis, including Kaplan-Meier estimation and log-rank tests or Cox proportional hazards models, further supported our findings. The analysis revealed significant differences in survival outcomes between different gene expression groups, indicating the potential impact of autophagy-related gene expression on breast cancer prognosis. Hazard ratios and confidence intervals were calculated to quantify the effect size of autophagy-related gene expression. Applying summary effects to the results of Cox regression analysis, we observed a range of Fisher r-to-z transformed coefficients, with the majority of estimates being positive. However, the average outcome did not significantly differ from zero, suggesting that the overall effect of autophagy-related genes on breast cancer prognosis may be influenced by heterogeneous outcomes across studies. Sensitivity analyses were conducted to evaluate the robustness of the results, and the absence of outliers or influential studies further supported the reliability of our findings. Considering the significance

of the identified genes and pathways, as well as the statistical considerations, our study provides valuable insights into the role of autophagy-related genes in breast cancer prognosis. These findings contribute to the understanding of the underlying mechanisms and may have implications for future research and clinical applications. However, it is important to acknowledge the limitations of our study, such as the reliance on publicly available datasets and the need for further validation in independent cohorts.

In conclusion, our study highlights the importance of autophagy-related genes in breast cancer prognosis and emphasizes their potential as prognostic markers or therapeutic targets. Future studies focusing on the functional characterization of specific autophagy-related genes and their interactions within the pathways will further enhance our understanding of their role in breast cancer progression and provide opportunities for personalized treatment strategies.

5. REFERENCES

1. Levy JM, Towers CG, Thorburn A. Targeting autophagy in cancer. Nature Reviews Cancer. 2017 Sep;17(9):528-42.
2. Cotzomi-Ortega I, Aguilar-Alonso P, Reyes-Leyva J, Maycotte P. Autophagy and its role in protein secretion: implications for cancer therapy. Mediators of Inflammation. 2018 Oct;2018.
3. Yeo S.K., Guan J.-L. Breast Cancer: Multiple Subtypes within a Tumor? *Trends Cancer.* 2017;3:753–760. doi: 10.1016/j.trecan.2017.09.001.
4. Prat A., Perou C.M. Deconstructing the Molecular Portraits of Breast Cancer. *Mol. Oncol.* 2011;5:5–23. doi: 10.1016/j.molonc.2010.11.003.
5. Lim E., Vaillant F., Wu D., Forrest N.C., Pal B., Hart A.H., Asselin-Labat M.-L., Gyorki D.E., Ward T., Partanen A., et al. Aberrant Luminal Progenitors as the

Candidate Target Population for Basal Tumor Development in BRCA1 Mutation Carriers. *Nat. Med.* 2009;15:907–913. doi: 10.1038/nm.2000.

6. Lim E., Vaillant F., Wu D., Forrest N.C., Pal B., Hart A.H., Asselin-Labat M.-L., Gyorki D.E., Ward T., Partanen A., et al. Aberrant Luminal Progenitors as the Candidate Target Population for Basal Tumor Development in BRCA1 Mutation Carriers. *Nat. Med.* 2009;15:907–913. doi: 10.1038/nm.2000.
7. Szymiczek A., Lone A., Akbari M.R. Molecular Intrinsic versus Clinical Subtyping in Breast Cancer: A Comprehensive Review. *Clin. Genet.* 2020 doi: 10.1111/cge.13900.
8. Kennecke H., Yerushalmi R., Woods R., Cheang M.C.U., Voduc D., Speers C.H., Nielsen T.O., Gelmon K. Metastatic Behavior of Breast Cancer Subtypes. *J. Clin. Oncol.* 2010;28:3271–3277. doi: 10.1200/JCO.2009.25.9820.
9. Lehmann B.D., Bauer J.A., Chen X., Sanders M.E., Chakravarthy A.B., Shyr Y., Pietenpol J.A. Identification of Human Triple-Negative Breast Cancer Subtypes and Preclinical Models for Selection of Targeted Therapies. *J. Clin. Investig.* 2011;121:2750–2767. doi: 10.1172/JCI45014.
10. Van Maaren M.C., de Munck L., Strobbe L.J.A., Sonke G.S., Westenend P.J., Smidt M.L., Poortmans P.M.P., Siesling S. Ten-Year Recurrence Rates for Breast Cancer Subtypes in the Netherlands: A Large Population-Based Study. *Int. J. Cancer.* 2019;144:263–272. doi: 10.1002/ijc.31914.
11. Li M., Liu J., Li S., Feng Y., Yi F., Wang L., Wei S., Cao L. Autophagy-Related 7 Modulates Tumor Progression in Triple-Negative Breast Cancer. *Lab. Investig.* 2019;99 doi: 10.1038/s41374-019-0249-2.
12. Tavera-Mendoza L.E., Westerling T., Libby E., Marusyk A., Cato L., Cassani R., Cameron L.A., Ficarro S.B., Marto J.A., Klawitter J., et al. Vitamin D Receptor Regulates Autophagy in the Normal Mammary Gland and in Luminal Breast Cancer Cells. *Proc. Natl. Acad. Sci. USA.* 2017;114:E2186–E2194. doi: 10.1073/pnas.1615015114.

13. Liang X.H., Jackson S., Seaman M., Brown K., Kempkes B., Hibshoosh H., Levine B. Induction of Autophagy and Inhibition of Tumorigenesis by Beclin 1. *Nature.* 1999;402:672–676. doi: 10.1038/45257
14. Smit L., Berns K., Spence K., Ryder W.D., Zeps N., Madiredjo M., Beijersbergen R., Bernards R., Clarke R.B. An Integrated Genomic Approach Identifies That the PI3K/AKT/FOXO Pathway Is Involved in Breast Cancer Tumor Initiation. *Oncotarget.* 2016;7:2596–2610. doi: 10.18632/oncotarget.6354.
15. Schäffner I., Minakaki G., Khan M.A., Balta E.-A., Schlötzer-Schrehardt U., Schwarz T.J., Beckervordersandforth R., Winner B., Webb A.E., DePinho R.A., et al. FoxO Function Is Essential for Maintenance of Autophagic Flux and Neuronal Morphogenesis in Adult Neurogenesis. *Neuron.* 2018;99:1188–1203.e6. doi: 10.1016/j.neuron.2018.08.017.
16. Audesse A.J., Dhakal S., Hassell L.-A., Gardell Z., Nemtsova Y., Webb A.E. FOXO3 Directly Regulates an Autophagy Network to Functionally Regulate Proteostasis in Adult Neural Stem Cells. *PLoS Genet.* 2019;15:e1008097. doi: 10.1371/journal.pgen.1008097.
17. Kenific C.M., Stehbens S.J., Goldsmith J., Leidal A.M., Faure N., Ye J., Wittmann T., Debnath J. NBR1 Enables Autophagy-Dependent Focal Adhesion Turnover. *J. Cell Biol.* 2016;212:577–590. doi: 10.1083/jcb.201503075.
18. Marsh T., Kenific C.M., Suresh D., Gonzalez H., Shamir E.R., Mei W., Tankka A., Leidal A.M., Kalavacherla S., Woo K., et al. Autophagic Degradation of NBR1 Restricts Metastatic Outgrowth during Mammary Tumor Progression. *Dev. Cell.* 2020;52:591–604.e6. doi: 10.1016/j.devcel.2020.01.025.
19. Katsuragi Y., Ichimura Y., Komatsu M. Regulation of the Keap1–Nrf2 Pathway by P62/SQSTM1. *Curr.Opin.Toxicol.* 2016;1:5461.doi: 10.1016/j.cotox.2016.09.005.
20. Jiang X., Bao Y., Liu H., Kou X., Zhang Z., Sun F., Qian Z., Lin Z., Li X., Liu X., et al. VPS34 Stimulation of P62 Phosphorylation for Cancer Progression. *Oncogene.* 2017;36:6850–6862. doi: 10.1038/onc.2017.295.

21. Chen HT, Liu H, Mao MJ, Tan Y, Mo XQ, Meng XJ, Cao MT, Zhong CY, Liu Y, Shan H, Jiang GM. Crosstalk between autophagy and epithelial-mesenchymal transition and its application in cancer therapy. Molecular cancer. 2019 Dec;18(1):1-9.
22. Benbrook DM, and Long A. Integration of autophagy, proteasomal degradation, unfolded protein response and apoptosis. Exp. Oncol. 2012; 34(3):286-97.
23. Yang Z, and Klionsky DJ. Mammalian autophagy: core molecular machinery and signaling regulation. Curr. Opin. Cell Biol. 2009; 22(2):124-31.
24. Polson HEJ, de Lartigue J, Rigden DJ, Reedijk M, Urbé S, Clague MJ, and Tooze SA. Mammalian Atg18 (WIPI2) localizes to omegasome-anchored phagophores and positively regulates LC3 lipidation. Autophagy 2010; 6(4):506-22.
25. Ohashi Y, and Munro S. Membrane delivery to the yeast autophagosome from the Golgi-endosomal system. Mol. Biol. Cell 2010; 21(22):3998-4008.
26. Abounit K, Scarabelli TM, and McCauley RB. Autophagy in mammalian cells. World J Biol Chem 2012; 3(1):1-6.
27. A. Chiarini, D. Bouvard, D. Arnoult, Autophagy Mechanism, Regulation, Functions, and Disorders, Volume 2012, doi.org/10.5402/2012/927064
28. Cuervo AM, and Wong E. Chaperone-mediated autophagy: roles in disease and aging. Cell Res. 2013; 24
29. . Mijaljica D, Prescott M, and Devenish RJ. Microautophagy in mammalian cells: revisiting a 40-year-old conundrum. Autophagy 7: 673–682, 2011
30. [6] Hamurcu Z., Delibasi N., Gecene S., Sener E.F., Donmez-Altuntas H., Ozkul Y., Canatan H., Ozpolat B. Targeting LC3 and Beclin-1 autophagy genes suppresses proliferation, survival, migration and invasion by inhibition of Cyclin-D1 and uPAR/Integrin beta1/Src signaling in triple negative breast cancer cells. J. Cancer Res. Clin. Oncol. 2018;144:415–430. doi: 10.1007/s00432-017-2557-5.
31. [7] Mathew R, Karp CM, Beaudoin B, Vuong N, Chen G, Chen HY, et al Autophagy suppresses tumorigenesis through elimination of p62. Cell 2009;137:1062–75.
32. [8] Qu X, Yu J, Bhagat G, Furuya N, Hibshoosh H, Troxel A, et al Promotion of tumorigenesis by heterozygous disruption of the beclin 1 autophagy gene. J Clin Invest 2003;112:1809–20.
33. [9] Hamurcu Z., Delibasi N., Gecene S., Sener E.F., Donmez-Altuntas H., Ozkul Y., Canatan H., Ozpolat B. Targeting LC3 and Beclin-1 autophagy genes suppresses proliferation,

survival, migration and invasion by inhibition of Cyclin-D1 and uPAR/Integrin beta1/Src signaling in triple negative breast cancer cells. J. Cancer Res. Clin. Oncol. 2018;144:415–430. doi: 10.1007/s00432-017-2557-5.

34. 76. Hashimoto I., Koizumi K., Tatematsu M., Minami T., Cho S., Takeno N., Nakashima A., Sakurai H., Saito S., Tsukada K., et al. Blocking on the CXCR4/mTOR signalling pathway induces the anti-metastatic properties and autophagic cell death in peritoneal disseminated gastric cancer cells. Eur. J. Cancer. 2008;44:1022–1029. doi: 10.1016/j.ejca.2008.02.043.
35. Yang S, Wang X, Contino G, Liesa M, Sahin E, Ying H, et al Pancreatic cancers require autophagy for tumor growth. Genes Dev 2011;25:717–29.
36. Li J, Yang B, Zhou Q, Wu Y, Shang D, Guo Y, et al. Autophagy promotes hepatocellular carcinoma cell invasion through activation of epithelial-mesenchymal transition. Carcinogenesis. 2013;34:1343–51.
37. White E., DiPaola R.S. The double-edged sword of autophagy modulation in cancer. Clin. Cancer Res. 2009;15:5308–5316. doi: 10.1158/1078-0432.CCR-07-5023.
38. Degenhardt K., Mathew R., Beaudoin B., Bray K., Anderson D., Chen G., Mukherjee C., Shi Y., Gelinas C., Fan Y., et al. Autophagy promotes tumor cell survival and restricts necrosis, inflammation, and tumorigenesis. Cancer Cell. 2006;10:51–64. doi: 10.1016/j.ccr.2006.06.001.
39. Macintosh RL, Timpson P, Thorburn J, Anderson KI, Thorburn A, Ryan KM. Inhibition of autophagy impairs tumor cell invasion in an organotypic model. Cell Cycle. 2012;11(10):2022–9.
40. Bartesaghi S, Faccenda D, Shaked-Rabi M, Sanzone S, McEvoy A, et al. The autophagy-associated factors DRAM1 and p62 regulate cell migration and invasion in glioblastoma stem cells. Oncogene. 2013;32:699–712.
41. Maycotte P, Jones KL, Goodall ML, Thorburn J, Thorburn A. Autophagy supports breast cancer stem cell maintenance by regulating IL6 secretion. Mol Cancer Res. 2015;13:651–8.
42. Sharif T, Martell E, Dai C, Kennedy BE, Murphy P, Clements DR, et al. Autophagic homeostasis is required for the pluripotency of cancer stem cells. Autophagy. 2017;13:264–84.

43. Lu Z, Luo RZ, Lu Y, Zhang X, Yu Q, Khare S, et al The tumor suppressor gene ARHI regulates autophagy and tumor dormancy in human ovarian cancer cells. J Clin Invest 2008;118:3917–29.
44. [14] Alvarez-Meythaler JG, Garcia-Mayea Y, Mir C, Kondoh H, LLeonart ME. Autophagy takes center stage as a possible cancer hallmark. Frontiers in Oncology. 2020 Oct 22;10:586069.
45. [15] Mowers EE, Sharifi MN, Macleod KF. Autophagy in cancer metastasis. Oncogene. 2017 Mar;36(12):1619-30.
46. [16] Bustos SO, Antunes F, Rangel MC, Chammas R. Emerging autophagy functions shape the tumor microenvironment and play a role in cancer progression-implications for cancer therapy. Frontiers in Oncology. 2020 Nov 25;10:606436.
47. Laddha SV, Ganesan S, Chan CS, White E. Mutational landscape of the essential autophagy gene BECN1 in human cancers. Mol Cancer Res. 2014;**12**:485–490.
48. Oh S, Xiaofei E, Ni D, Pirooz SD, Lee JY, Lee D, Zhao Z, Lee S, Lee H, Ku B, et al. Downregulation of autophagy by Bcl-2 promotes MCF7 breast cancer cell growth independent of its inhibition of apoptosis. Cell Death Differ. 2011;**18**:452–464.
49. Huo Y, Cai H, Teplova I, Bowman-Colin C, Chen G, Price S, Barnard N, Ganesan S, Karantza V, White E, et al. Autophagy opposes p53-mediated tumor barrier to facilitate tumorigenesis in a model of PALB2-associated hereditary breast cancer. Cancer Discov. 2013;**3**:894–907.
50. Cancer Genome Atlas Network. Comprehensive molecular portraits of human breast tumours. Nature. 2012**;490**:61–70.
51. 58. Won KY, Kim GY, Kim YW, Song JY, Lim SJ. Clinicopathologic correlation of beclin-1 and bcl-2 expression in human breast cancer. Hum Pathol. 2010;**41**:107–112.
52. Wei H., Wei S., Gan B., Peng X., Zou W., Guan J.-L. Suppression of Autophagy by FIP200 Deletion Inhibits Mammary Tumorigenesis. *Genes Dev.* 2011;25:1510–1527. doi: 10.1101/gad.2051011.
53. Hamurcu Z., Delibaşı N., Geçene S., Şener E.F., Dönmez-Altuntaş H., Özkul Y., Canatan H., Ozpolat B. Targeting LC3 and Beclin-1 Autophagy Genes Suppresses

Proliferation, Survival, Migration and Invasion by Inhibition of Cyclin-D1 and UPAR/Integrin B1/ Src Signaling in Triple Negative Breast Cancer Cells. *J. Cancer Res. Clin. Oncol.* 2018;144:415–430. doi: 10.1007/s00432-017-2557-5.

54. Al-Hajj M, Wicha MS, Benito-Hernandez A, Morrison SJ andClarke MF: Prospective identification of tumorigenic breast
cancer cells. Proc Natl Acad Sci USA 100: 3983-3988, 2003.
55. Abdal Dayem A, Choi HY, Yang GM, Kim K, Saha SK and Cho SG: The anti-cancer effect of polyphenols against breast cancer and cancer stem cells: Molecular mechanisms. Nutrients 8: 581-618, 2016.
56. Chang N.C. Autophagy and Stem Cells: Self-Eating for Self-Renewal. *Front. Cell Dev. Biol.* 2020;8:138. doi: 10.3389/fcell.2020.00138.
57. Ginestier C., Hur M.H., Charafe-Jauffret E., Monville F., Dutcher J., Brown M., Jacquemier J., Viens P., Kleer C.G., Liu S., et al. ALDH1 Is a Marker of Normal and Malignant Human Mammary Stem Cells and a Predictor of Poor Clinical Outcome. *Cell Stem Cell.* 2007;1:555–567. doi: 10.1016/j.stem.2007.08.014.
58. Maycotte P., Jones K.L., Goodall M.L., Thorburn J., Thorburn A. Autophagy Supports Breast Cancer Stem Cell Maintenance by Regulating IL6 Secretion. *Mol. Cancer Res.* 2015;13:651–658. doi: 10.1158/1541-7786.MCR-14-0487.
59. Mowers EE, Sharifi MN and Macleod KF: Autophagy in cancer metastasis. Oncogene 36: 1619-1630, 2017
60. Yao D, Wang P, Zhang J, Fu L, Ouyang L and Wang J:Deconvoluting the relationships between autophagy and metastasis for potential cancer therapy. Apoptosis 21: 683-698,2016.
61. Han Q, Deng Y, Chen S, Chen R, Yang M, Zhang Z, Sun X,Wang W, He Y, Wang F, *et al*: Downregulation of ATG5-dependent macroautophagy by chaperone-mediated autophagy promotes breast cancer cell metastasis. Sci Rep 7: 4759, 2017.
62. 90. Maycotte P., Jones K.L., Goodall M.L., Thorburn J., Thorburn A. Autophagy Supports Breast Cancer Stem Cell Maintenance by Regulating IL6 Secretion. *Mol. Cancer Res.* 2015;13:651–658. doi: 10.1158/1541-7786.MCR-14-0487.

63. Wei H., Wei S., Gan B., Peng X., Zou W., Guan J.-L. Suppression of Autophagy by FIP200 Deletion Inhibits Mammary Tumorigenesis. *Genes Dev.* 2011;25:1510–1527. doi: 10.1101/gad.2051011.
64. 100. Sosa M.S., Bragado P., Aguirre-Ghiso J.A. Mechanisms of Disseminated Cancer Cell Dormancy: An Awakening Field. *Nat. Rev. Cancer*. 2014;14:611–622. doi: 10.1038/nrc3793.
65. 105. Smith A.G., Macleod K.F. Autophagy, Cancer Stem Cells and Drug Resistance. *J. Pathol.* 2019;247:708–718. doi: 10.1002/path.5222.
66. 97. Vera-Ramirez L., Vodnala S.K., Nini R., Hunter K.W., Green J.E. Autophagy Promotes the Survival of Dormant Breast Cancer Cells and Metastatic Tumour Recurrence. *Nat. Commun.* 2018;9:1944. doi: 10.1038/s41467-018-04070-6.
67. Zhang W., Bado I., Wang H., Lo H.-C., Zhang X.H.-F. Bone Metastasis: Find Your Nicheand Fit In. *Trends Cancer.* 2019;5:95–110. doi: 10.1016/j.trecan.2018.12.004.
68. Bincoletto C, Bechara A, Pereira GJS, Santos CP, Antunes F, Peixoto da-Silva J, Muler M, Gigli RD, Monteforte PT, Hirata H, et al: Interplay between apoptosis and autophagy, a challenging puzzle: New perspectives on antitumor chemotherapies. Chem Biol Interact. 206:279–288. 2013.
69. Lu C, Wang W, Jia Y, Liu X, Tong Z and Li B: Inhibition of AMPK/autophagy potentiates parthenolide-induced apoptosis in human breast cancer cells. J Cell Biochem. 115:1458–1466. 2014.
70. Vinayak S and Carlson RW: mTOR inhibitors in the treatment of breast cancer. Oncology (Williston Park). 27:38–44, 46, 48 passim. 2013.PubMed/NCBI
71. Petherick KJ, Williams AC, Lane JD, Ordóñez-Morán P,Huelsken J, Collard TJ, Smartt HJ, Batson J, Malik K,Paraskeva C, *et al*: Autolysosomal β-catenin degradation regulatesWnt-autophagy-p62 crosstalk. EMBO J 32: 1903-1916, 2013.
72. Wang Y, Han C, Lu L, Magliato S and Wu T: Hedgehog signaling pathway regulates autophagy in human hepatocellular carcinoma cells. Hepatology 58: 995-1010, 2013.
73. . Zhou X, Yue GG, Chan AM, Tsui SK, Fung KP, Sun H, Pu J and Lau CB: Eriocalyxin B, a novel autophagy inducer, exerts antitumor activity through the suppression of

Akt/mTOR/p70S6K signaling pathway in breast cancer. Biochem Pharmacol 142:58-70, 2017.

74. Wong VKW, Zeng W, Chen J, Yao XJ, Leung ELH, Wang QQ,Chiu P, Ko BCB and Law BYK: Tetrandrine, an activator of autophagy, induces autophagic cell death via PKC-α inhibition and mTOR-dependent mechanisms. Front Pharmacol 8: 351,2017.
75. Han H, Li J, Feng X, Zhou H, Guo S and Zhou W: Autophagyrelated genes are induced by histone deacetylase inhibitor suberoylanilide hydroxamic acid via the activation of cathepsin B in human breast cancer cells. Oncotarget 8:53352-53365, 2017.
76. Cufí S., Vazquez-Martin A., Oliveras-Ferraros C., Corominas-Faja B., Cuyàs E., López-Bonet E., Martin-Castillo B., Joven J., Menendez J.A. The Anti-Malarial Chloroquine Overcomes Primary Resistance and Restores Sensitivity to Trastuzumab in HER2-Positive Breast Cancer. *Sci. Rep.* 2013;3:2469. doi: 10.1038/srep02469.
77. Cook KL, Shajahan AN, Clarke R. Autophagy and endocrine resistance in breast cancer. Expert Rev Anticancer Ther. 2011;11:1283–1294.
78. 63. Samaddar JS, Gaddy VT, Duplantier J, Thandavan SP, Shah M, Smith MJ, Browning D, Rawson J, Smith SB, Barrett JT, et al. A role for macroautophagy in protection against 4-hydroxytamoxifen-induced cell death and the development of antiestrogen resistance. Mol Cancer Ther. 2008;7:2977–2987.
79. Nag S, Qin J, Srivenugopal KS, Wang M, Zhang R. The MDM2-p53 pathway revisited. J Biomed Res. 2013;27:254–271.
80. Lorin S, Hamaï A, Mehrpour M, Codogno P. Autophagy regulation and its role in cancer. Semin Cancer Biol. 2013;23:361–379.
81. Maycotte P, Aryal S, Cummings CT, Thorburn J, Morgan MJ, Thorburn A. Chloroquine sensitizes breast cancer cells to chemotherapy independent of autophagy. Autophagy. 2012;8:200–212.
82. Bristol ML, Emery SM, Maycotte P, Thorburn A, Chakradeo S, Gewirtz DA. Autophagy inhibition for chemosensitization and radiosensitization in cancer: do the preclinical data support this therapeutic strategy? J Pharmacol Exp Ther. 2013;344:544–552.
83. Bristol ML, Di X, Beckman MJ, Wilson EN, Henderson SC, Maiti A, Fan Z, Gewirtz DA. Dual functions of autophagy in the response of breast tumor cells to radiation: cytoprotective

autophagy with radiation alone and cytotoxic autophagy in radiosensitization by vitamin D 3. Autophagy. 2012;8:739–753. [PMC free article] [PubMed] [Google Scholar]

84. Akar U, Chaves-Reyez A, Barria M, Tari A, Sanguino A, Kondo Y, Kondo S, Arun B, Lopez-Berestein G, Ozpolat B. Silencing of Bcl-2 expression by small interfering RNA induces autophagic cell death in MCF-7 breast cancer cells. Autophagy. 2008;4:669–679.

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