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Genome Analysis and Human Health

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Preface

Genome research has indeed pampered our optimism as it was construed that the same would enhance our understanding on the mechanisms that lead to genetic diseases. In the context of human health, genetics involves study on single gene and their regulation to improve public health and prevent diseases. Genetic research helps to identify diseases and health problems that are more likely to be influenced by genetic factors. Genetic tests enable the risk assessment and determine the predisposition of an individual to various diseases by uncovering the mutations or variations in the genome. Such information may be useful in managing an individual's lifestyle and healthcare system. In addition to testing for particular conditions, genetic research provides solutions to health problems caused by genetic abnormalities and mutations either by medications or genetic modification. Most genetic disorders cannot be cured; however, many people have restored their health and avoided potentially life-threatening diseases with the help of genetic research by taking due precautions coupled with advanced medicaments and changed lifestyle.

Continuous technological improvements in DNA sequencing have created an ambiance par excellence that a large number of disease-causing microbe and viral genomes are sequenced on regular basis. The availability and the integration of genetical information have been the driving forces toward our understanding of the normal and abnormal genomes.

We believe that newer and far more despicable diseases would continue to emerge so also the quest to fight these diseases. Conceptually, advances in genetical knowledge fueled by technology could be used to prevent diseases creating much healthier gene pool. Thus, genome analysis both for normal and diseased ones would continue to upgrade our knowledge ensuring hope and assuring a healthy world.

New Delhi, India

Leena Rawal
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Introduction

A human genome contains approximately 3.3 billion DNA bases. By comparing an individual's sequence to a human genome reference sequence, DNA changes are detected at almost every DNA base position. Depending on the location, changes in the DNA bases may or may not alter the gene functions. Even if it does not affect a gene, the changes may still affect the genetic structure of an individual. The data gained from the genome sequence is subjected to various bioinformatics annotation tools and analyzed so as to decipher the core reasons behind these DNA changes or variations that may have an impact on individuals' health. Some of the DNA changes identified in the sequence are linked to genetic disorders that can be inherited within a family. These variations can affect the molecular pathways of the cell, leading to alterations in the physical trait, or can be linked to risk for common diseases.

With the advent of new high-throughput technologies, the conventional focus on genetics and single genes is drifting toward the study of the whole genome including the exome sequencing, the study of complex genes, gene–gene interactions, and the association between genes and environment (epigenetics). This evolution in genomics, genetics, and other related molecular biology technologies has created substantial avenues for the advanced understanding, prevention, treatment, and cure of human diseases.

This book is intended to provide basic information on genome analysis and its impact on human health. It focuses on different approaches that have been adopted to address one or the other issues related to human health including cancer. Additionally, it covers the domain that still must be explored in order to understand the signaling processes in the genome and gene–gene interactions encompassing a large number of still undefined and poorly understood interactomes that affect human health.

About the Editors

Sher Ali is an internationally respected scientist in the field of genomics and molecular genetics. He received his PhD from the University of Delhi in 1981 and subsequently worked as an Alexander von Humboldt fellow at the Max Planck Institute of Immunobiology in Freiburg, Germany. He retired from the National Institute of Immunology (NII), New Delhi, in 2015 after serving the institute for more than 25 years. At the NII, he was head of the Molecular Genetics Laboratory. Currently, he is working as a professor at the Centre for Interdisciplinary Research in Basic Sciences, Jamia Millia Islamia, New Delhi.

His research interests include genome analysis, molecular human and animal genetics, germ line genetics, toxicogenomics, DNA diagnosis, and cancer genetics. His outstanding research has attracted attention from various pharmaceutical companies. Dr. Ali has published over 100 peer-reviewed papers in reputed international journals. He is a member of all three academies of sciences in India, namely, the Indian Academy of Sciences, National Academy of Sciences, and Indian National Science Academy, and has been a reviewer and editorial board member for various prestigious journals such as *Molecular and Cellular Probes*, *American Journal of Cancer Genetics*, *Scientific Reports*, etc. He is the recipient of several national and international awards, including most recently an Alexander von Humboldt-Stiftung fellowship in Bonn, Germany, and the N.K. Iyengar Memorial Gold Medal.

Leena Rawal is currently working as a senior scientist at the Department of Cytogenetics, Dr. Lal PathLabs Limited, New Delhi. She was awarded PhD degree in Molecular Genetics by the University of Delhi in 2015 and subsequently worked as a postdoctoral fellow at the National Institute of Immunology, New Delhi. She has since worked as a senior scientist and head of the Molecular Diagnostics and Research Division at a renowned pharmacogenomics-based organization. Dr. Rawal has published several original papers in international peer-reviewed journals like *PLOS ONE*, *BMC Genomics*, *DNA and Cell Biology*, *Gene*, *Journal of Cellular Biochemistry* and the *Journal of Biomolecular Structure and Dynamics*, etc. Her expertise lies in the area of human and animal genetics, proteomics, cytogenetics, and bioinformatics. She has presented her work at various national and international platforms.

Genetic and Epigenetic Regulation of Autophagy in Cancer

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Anup S. Pathania, Ubaid S. Makhdoomi, and Fayaz A. Malik

1.1 Introduction

Cancer is a class of disease characterized by cells' abnormal growth and division. Cancer cells grow very fast in an uncontrolled manner as compared to normal cells and form lumps or tissue mass called tumor (except leukemia). Solid tumors are benign in nature as long as they are localized to their tissue of origin and become malignant when cells migrate to distant vital tissues of the body like, brain, bone, liver, lung, etc. through blood or lymphatic system. The transformation of normal cells into cancerous cells is a multistep process caused by mutations. The process of accumulating mutations normally takes many years, and several mutations are needed for a normal cell to acquire such oncogenic behavior. In all cancers, these mutations are mainly found in tumor suppressor and proto-oncogenes. Mutations in tumor suppressor genes render them with loss of functionality and inactivate their inhibitory properties on cell growth and division. Such mutations are also known as loss of function mutations and they are common in cancer cells. Some common examples of tumor suppressor genes bearing such mutations in cancer cells are retinoblastoma gene (RB), p53, BRCA (breast cancer genes), APC (adenomatous polyposis coli), PTEN (phosphatase and tensin homologue), p27, etc. (Lee and Muller 2010). In normal cells, regulated counterparts of oncogenes are known as proto-oncogenes that control cell division and proliferation. Mutations in proto-oncogenes deregulate their activities (also known as gain in function) leading their conversion into the cancer-forming

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oncogenes like those of RAS, Myc, HER2, Cyclin D, Bcl-2, etc. (Lee and Muller 2010). Many such genetic changes that happened are like point mutations, insertions, deletions, gene amplifications, or chromosomal translocation of proto-oncogenes to another normal gene that dysregulate its expression. The factors behind these genetic changes are random and are not cell specific and are different in different types of cancers, e.g., lung cancer, 90% of cases are associated with cigarette smoking and risk of cancer increase with tobacco dose and is the reason for the mutations in lung cells (Sasco et al. 2004). Similarly, the use of alcohol, tobacco, and human papillomavirus or Epstein-Barr virus infection are important risk factors for head and neck carcinomas (Goldenberg et al. 2004; Leemans et al. 2011). The presence of carcinogens like heterocyclic amines (HCAs), N-nitroso compounds (NOCs), and heme in red meat damages the DNA of cells that line the digestive system (Alexander and Cushing 2011). However, it is still to be understood the reasons behind the acquisition of mutations in key genes of normal cells which become nonresponsive to cellular homeostasis. A nonsmoker can develop a lung cancer; women who take normal food and exercise regularly with no genetic history of breast cancer can have this type of tumor. As our understanding of cancer is continuously growing, it has been established that besides environmental factors, genetic predisposition also plays a major role in cancer development. Genetic predisposition means increase in the likelihood of developing a particular disease based on the genetic makeup of person he or she acquired from his parents or ancestors. Mammalian cells have two copies of genes, and as long as cells contain at least one functional copy, the gene remains fully functional. The examples of such genetic mutations in mammalian cells include the retinoblastoma gene (RB), p53, BRCA1 and BRCA2 in breast cancer, TERT in melanoma, APC in colon cancer, etc. However, it is to mention that more than 75% of cancers are sporadic, which means they occur by chance and have no familial history. Apart from genetic defects, influence of epigenetic changes is also equally responsible for cancer development and progression. Though epigenetic alterations do not include any changes in cellular DNA sequences, they are mitotically and meiotically inheritable. Epigenetic changes can switch on or off the genes by controlling their transcription. The common epigenetic events occur in cells include methylation of cytosine bases of DNA present within CpG dinucleotides that are found in 5′-end regulatory regions of many genes and almost in all housekeeping genes. Similarly, acetylation or deacetylation occurs on the lysine residues present within N-terminal tail of histone core of the nucleosome as a part of gene regulation. Small noncoding regulatory RNAs (siRNAs) also play a critical role in tumorigenesis. In this chapter, we will try to discuss the role of genetic and epigenetic changes associated with the process of autophagy and its implications in cancer. Autophagy is a catabolic process that uses cell lysosomal machinery to degrade unnecessary or dysfunctional cellular constituents. This process not only eliminates the damaged organelles but also provides raw materials to the cells under stress-related conditions to maintain homeostasis. Autophagy has been shown to play an important role in cancer progression (Choi 2012), angiogenesis

(Du et al. 2012), epithelial to mesenchymal transition (Li et al. 2013), metastasis (Peng et al. 2013), resistance (Peng et al. 2013), inflammation (Levine et al. 2011), infection (Deretic 2010), and neurological disorders (Nixon and Yang 2012). However, prolonged autophagy activation may also lead to cell death due to excessive catabolism, known as type II programmed cell death or autophagic cell death.

1.2 The Process of Autophagy

Autophagy starts with the formation of membranous structures or phagophore around the cytoplasmic sites known as pre-autophagosomal structure (PAS) discovered in yeast (Klionsky 2007). Phagophore membrane formation is mainly contributed by ER, Golgi, and endosomes under the control of various signaling events. Sequential activation of specific autophagy-related genes (ATGs) is involved in the formation of phagophore. ATGs were first identified in yeast through genetic screening, and many of their homologues have been subsequently found and characterized in higher eukaryotes. Autophagic process mainly involved five events, induction, vesicle nucleation, vesicle elongation, autophagosomes formation, and autophagosome-lysosome fusion. The main regulator of autophagy induction in cells is the mammalian target of rapamycin (mTOR) (Jung et al. 2009), a nutrient sensor of the cell that inhibits autophagy under nutrient-rich conditions. mTOR phosphorylates autophagy protein ATG13L at multiple serine residues and mammalian homologues of ATG1 protein ULK-1 or ULK-2 (UNC-51-like kinases). mTOR-mediated phosphorylation of ULK-1 and ULK-2 inhibits their activity, rendering them unable to phosphorylate and activate focal adhesion kinase family-interacting protein of 200 kD (FIP200). ULK-1, ULK-2, and ATG13 form a complex with FIP200 known as ATG13L-ULK-1/2-FIP200 complex which recruits other proteins for autophagosome formation. During nutrient starvation, cellular ATP levels decrease in cells; as a consequence of which, cAMP levels increase which further activates energy sensor protein, AMPK (AMP-activated protein kinase). AMPK inhibits mTOR activity and promotes hypophosphorylation of ATG13L which favors the formation of active ATG13L-ULK-1/2-FIP200 complex and hence the induction of autophagy (Jung et al. 2009). Vesicle nucleation starts with the recruitment of ATGs to the growing phagophore. Although the process is less understood, type III PI3 kinases play an important role in the recruitment of ATGs to the phagophore. Type III PI3 kinases consist of single catalytic subunit VPS34 (homologue of yeast vacuolar protein sorting-associated protein 34) and produce PtdIns3P (phosphatidylinositol 3-phosphate). VPS34 interacts with autophagic proteins Beclin and VPS15 and forms type III PI3 kinase complex, also known as Beclin-VPS34-VPS15 complex. This complex is recruited to PAS by ATG14 where it acts as a localization site for most ATG proteins that facilitate autophagosome formation (Mizushima et al. 2011). The third step, vesicle elongation, involves the expansion of

phagophore membranes around PAS for the formation of autophagosomes. Two ubiquitin-like conjugation systems, ATG5-ATG12 and ATG8 are involved in this process (Nakatogawa 2013). Eloborating these systems is beyond the scope of this chapter. However, these interactions lead to the lipidation of ATG8 (LC3B in mammals), a typical marker of autophagy, with phosphatidylethanolamine (PE) that insets ATG8 into autophagosome membranes. Once the autophagosomes are formed, they fuse with lysosomes to form autolysosomes in which the autophagic substrates are degraded and recycled (Fig. 1.1).

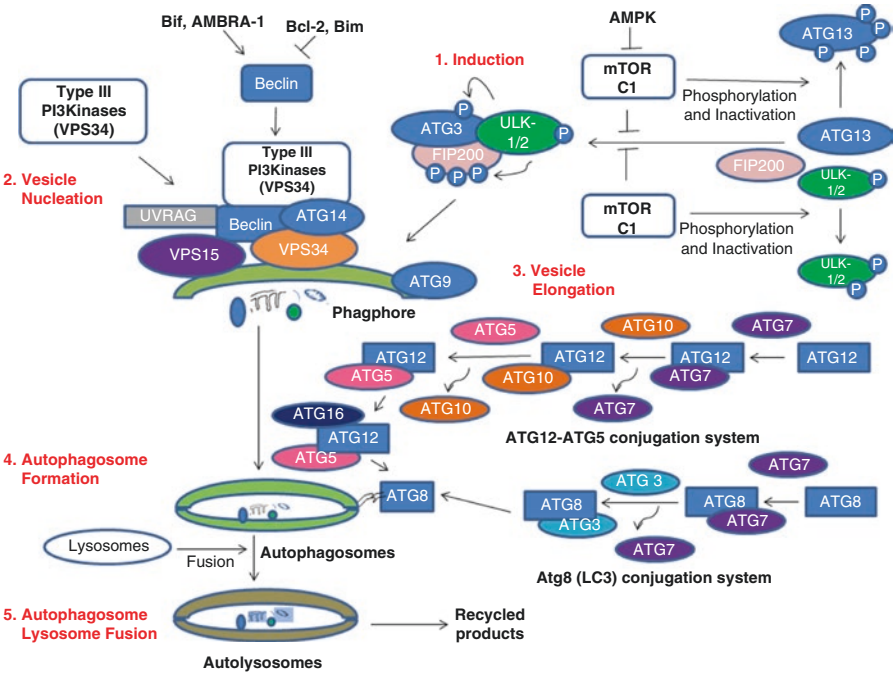

Fig. 1.1 The autophagic process. Formation of autophagosomes involves the various steps. The process begins with the formation of double membrane structures (phagophores) around the cargo molecules (damaged organelles and macromolecules, misfolded proteins, pathogens, etc.) in the cytoplasm. The process is induced by formation of ATG13-ULK-1/2-FIP200 complex at the autophagosome assembly site in mammalian cells. mTOR promotes the phosphorylation and inactivation of ATG13 and ULK-1/2 under nutrient-rich conditions. Inhibition of mTOR triggers the formation of this complex and induces autophagy in cells. Next step is vesicle nucleation which is performed by type III PI3 kinases along with Beclin and VPS15. Type III PI3 kinase subunit, VPS34, interacts with autophagic proteins Beclin and VPS15 and forms Beclin-VPS34-VPS15 complex which is recruited to the phagophore by ATG14. Beclin-VPS34-VPS15 complex acts as a nucleation site for most of ATGs involved in autophagosome formation. Vesicle elongation involves two ubiquitin-like conjugation systems, ATG5-ATG12 conjugation systems and ATG8 (LC3B in mammals) conjugation systems, which promote the lipidation of LC3B with phosphatidyl ethanolamine and complete the formation of autophagosomes. Once autophagosomes are formed, it fused with lysosomes to form autolysosomes which degrade its inner constituents via lysosomal hydrolases

1.3 Autophagy in Cancer

Autophagy dysregulation is a common phenomenon in almost all cancers, and modulating this process is an area of great interest in cancer drug discovery. The expression of many autophagy-associated genes is altered in cancer cells that leads to tumor progression. The first direct link between autophagy and cancer was established in early 1999 by Aita et al., demonstrating that the mono-allelic deletions of beclin (an ortholog of yeast ATG6 present on chromosome 17q21) are found in human breast and ovarian carcinoma cell lines (Aita et al. 1999). The same year Liang and co-workers reported that beclin promotes autophagy in autophagy-defective yeast having targeted the disruption of *agp6/vps30* and in breast cancer cell line MCF-7 (Liang et al. 1999). Beclin-induced autophagy inhibits cellular proliferation, clonogenic survival, and tumorigenesis in mice (Liang et al. 1999). Further work by Qu et al. (2003) demonstrated that heterozygous disruption of beclin increases cellular proliferation, spontaneous tumorigenesis, and development of HBV-induced premalignant lesions in mouse tumor models. Southern blots and mutational analysis of genomic DNA of beclin (+/−) mice did not reveal any deletions or rearrangements in the remaining normal beclin allele, and hence inactivation of only one allele is sufficient to promote tumorigenesis. These results show that Beclin is a haploinsufficient tumor suppressor gene and it does not follow Knudson two-hit hypothesis where mutations in both alleles are required for tumor suppressor gene to lose its function (Qu et al. 2003). These findings also revealed a new role of autophagy in preventing dysregulated growth of tumor cells besides maintaining homeostasis. Beclin is a Bcl-2 homology (BH)-3 domain-only protein localized throughout cytoplasm including mitochondria, ER, and nucleus (Kang et al. 2011). Beclin gene maps to a region of 150 kb centromeric to BRCA1 gene present on chromosome 17q21 and encodes a 2098-bp transcript, with a 120-bp 5′ UTR, 1353-bp coding region, and 625-bp 3′ UTR (Aita et al. 1999). Beclin contains three domains: N-terminal short BH3 domain (105–125 residues), a central coiled-coil segment (114–269), and C-terminal evolutionary conserved domain (244–337) (Sinha and Levine 2008; Huang et al. 2012). The Bcl-2 member proteins Bcl-2 and Bcl-xL regulate autophagy via binding to BH3 domain of Beclin and inhibit its association with class III PI3 kinases, an important autophagy regulator in cells (Pattingre et al. 2005; Ku et al. 2008). Furthermore, phosphorylation of BH3 domain at threonine 308 residue by proapoptotic kinase Mst1 stabilizes Beclin-Bcl-2 interactions and inhibits the formation of ATG14L-Beclin1-Vps34 autophagic complex (Maejima et al. 2013). Another proapoptotic BH3 only protein BCL2L11 (also known as BIM) inhibits autophagy in cells by interacting with Beclin and facilitates its binding with dynein protein DYNLL1. Starvation induces BIM phosphorylation through MAPK8/JNK pathway and abolishes BIM-DYNLL1 interactions, allowing dissociation of Beclin from BIM, and induces autophagy (Luo and Rubinsztein 2013). The central coiled-coil domain of Beclin is required for its interactions with autophagy proteins ATG14 and ultraviolet radiation resistance-associated gene (UVRAG) which forms Beclin-ATG14 or Beclin-UVRAG heterodimers during autophagy (Li et al. 2012). The third evolutionary conserved domain of Beclin is required for its binding with Vps34 and lipid membranes (Furuya et al. 2005; Huang et al. 2012). Mutations in this region hinder Beclin binding with membranes and compromise autophagy;

however, it has no effects on Beclin interactions with other autophagy mediators like UVRAG and ATG14 (Huang et al. 2012; Fu et al. 2013). Mutations in Beclin gene are found in many cancer types and have been associated with the poor prognosis. In prostate carcinoma tumor, low oxygen and androgen deprivation trigger AMPK activation which induces autophagy via beclin activation. The induced autophagy is protective in nature and its pharmacological or genetic inhibition induces apoptosis in these tumor cells (Chhipa et al. 2011). Furthermore, Beclin and its counterpart LC3B are involved in the pathogenesis of benign prostatic hyperplasia (BPH) cells and promote androgen independence in prostate cancer cells (Liu et al. 2013a).

1.4 Autophagy-Associated Genes: Mutations and Role in Cancer

There are 32 autophagy-related genes or ATG genes till discovered, out of which 18 ATGs are directly involved in autophagosome formation upon starvation (Mizushima et al. 2011). Most of ATG genes are evolutionary conserved between yeast and mammals. Somatic mutations in ATG genes are frequently observed in different cancers. Frameshift mutations in ATG2B, ATG5, ATG9B, and ATG12 are found in gastric and colorectal carcinomas (Kang et al. 2009). The frequency of such mutations is immense in carcinomas with high microsatellite instability as compared to those with low microsatellite instability. DNA sequence analysis of gastric and colorectal carcinoma patients found single-base deletion mutations in exon 20 of ATG2B and in exons 8 and 10 of ATG5, identical deletion mutations in exon 1-1, and three identical deletion mutations in exon 1-2 of *ATG9B* (Kang et al. 2009). Another autophagy gene, ATG12, is commonly mutated in breast cancer cells targeted against HER2-based therapies. ATG12 is upregulated in trastuzumab-resistant HER2-positive breast cancer cell lines as compared to trastuzumab-sensitive cell lines (Cufi et al. 2012). Quantitative real-time PCR-based arrays of 84 autophagy genes in trastuzumab-responsive SKBR3 and trastuzumab refractory JIMT1 breast cancer cell lines reveal the overexpression of ATG12 in JIMT1 cells as compared to SKBR3 (Cufi et al. 2012). Genetic knockdown of ATG12 by small hairpin RNA sensitizes breast JIMT1 cells to trastuzumab and HER1/HER2 tyrosine kinase inhibitors. Trastuzumab treatment showed strong tumor growth inhibitory effect in ATG12-shRNA/JIMT1 xenograft animal models as compared to wild-type ATG12 expressing JIMT xenografts (Cufi et al. 2012). Additionally, autophagy gene UVRAG is found to be mutated in many cancers (Liang and Jung 2010; Ionov et al. 2004). Frameshift mutations in UVRAG are present in colorectal and gastric carcinomas with high microsatellite instability (Kim et al. 2008). UVRAG is a Beclin-interacting protein that associates with Beclin-VPS34-VPS15 complex and promotes vesicular trafficking and autophagosome formation. UVRAG suppresses the proliferation and tumorigenicity in human colon cancer cells (Liang et al. 2006). Genetic silencing of UVRAG or Beclin by specific siRNA increases radiation-induced DNA double-stranded breaks and apoptotic cell death in 5-fluorouracil (5-FU)-treated irradiated colorectal cancer cells. UVRAG and Beclin interact with each other during DNA repair, and UVRAG mutants that are unable to bind to Beclin show the greater extent of DNA damage during irradiation as compared to normal UVRAG expressed cells. Furthermore, Beclin and UVRAG suppression increases centrosome number in cells that leads to spindle malformations and chromosome segregation errors (Park et al. 2014). Abnormal UVRAG expression along

with BRCA1, BECN1, CCND1, and PTEN genes has been associated with human breast carcinogenesis. The mRNA levels of these genes are downregulated in breast cancer cells as compared to normal breast tissues and linked with the pathogenesis of the disease (Wu et al. 2012). In non-medullary thyroid carcinoma (NMTC), genetic variations in autophagy genes increase susceptibility for cancer progression and outcome. NMTC patients show the statistically significant relation between ATG5 genetic variants and susceptibility for NMTC. G allele of the ATG5 rs2245214 SNP is mutated in NMTC patients and shows association with prognosis of the disease (Plantinga et al. 2014). Another important link between autophagy and thyroid carcinogenesis is single nucleotide polymorphism known as Thr300Ala polymorphism (threonine at position 300 is replaced by alanine, rs2241880) in ATG16L gene, which increases susceptibility for thyroid cancer. One possible mechanism for such association between ATG16L and thyroid cancer is the modulation of pro-inflammatory cytokine IL-1β by ATG16L which hinders its antiproliferative effect in thyroid cancer cells (Huijbers et al. 2012). Furthermore, Thr300Ala polymorphism in ATG16L is also associated with the increased risk of developing colorectal carcinoma, and patients carrying the less common GG genotype are at higher risk than those carrying more conmen AA genotype (Nicoli et al. 2014). Additionally, colorectal cancer patients show enhanced expression of ATG10, which is associated with lymphovascular invasion and lymph node metastasis. ATG10 is highly upregulated in patients with sporadic colorectal cancer and is involved in metastasis and tumor invasion (Jo et al. 2012). Patients who did not express ATG10 have significantly higher disease-free survival and overall survival rate than those bearing ATG10-expressing tumors. Colorectal carcinoma cell lines AMC5, LoVo, SW480, SW48, HCT15, DLD1, RKO, and CaCo2 show higher ATG10 expression as compared to normal colorectal cancer cell line CCD841. Silencing of ATG10 by using siRNA approach suppresses cell proliferation in HCT116 cells (Jo et al. 2012). Furthermore, mutations in 5q14 regions of ATG10 are found in ovarian (Ramus et al. 2003), gastric (Oga et al. 2001), and pancreatic cancer (Shiraishi et al. 2001). ATG10 linked two SNPs, rs1864182 and rs10514231, which are associated with risk factors in developing breast cancer. These genetic variants of ATG10 increase the susceptibility of breast cancer in Chinese population (Qin et al. 2013). Furthermore, ATG10 expression is upregulated in mesenchymal stem cells along with ATG12 and LC3B in serum-starved breast cancer cells. The increased expression of these proteins supports cell survival and growth by providing energy and secreting anti-apoptotic proteins (Sanchez et al. 2011). Serum starvation decreases the proliferation in breast cancer cell line MCF-7; however, its co-culture with normal or serum-starved mesenchymal stem cells increases their survival rate and proliferation. Inhibition of autophagy with autophagy inhibitors chloroquinone (CQ) and bafilomycin or by Beclin silencing decreases cell survival to a great extent signifying the protective role of autophagy during stress (Sanchez et al. 2011).

1.5 Genetic Regulation of Autophagy in Tumor Suppression and Promotion

After the discovery of Beclin having both tumor-suppressive and autophagy-inducing functions, many autophagy genes have been discovered, and mutations in these genes have been linked to tumor progression and growth. Mutations and deletions of three critical autophagic genes, ATG2A (1%), ATG7 (2%), and ATG13 (5%), are

found in nasopharyngeal carcinoma (NPC) patients (Lin et al. 2014). Although these changes are not significant, this is the first report about such genetic lesions in the ATG genes of cancer cells. Another important autophagy protein that has tumor-suppressive functions is ULK-1, a member of ATG13L-ULK-1/2-FIP200 complex. The mRNA and protein levels of ULK-1 are lower in breast cancer tissues as compared to matched normal tissues. Immunohistochemical staining of ULK-1 in 298 nonmetastatic invasive breast cancer tissues revealed lesser expression of ULK-1 in 70% of cases, whereas the adjacent noncancerous tissues have moderate to strong expression. The diminished ULK-1 expression is associated with reduced autophagic capacity and the progression of the disease (Tang et al. 2012). These findings also suggest the use of ULK-1 as a novel prognostic biomarker for breast cancer patients. ULK-1 and its counterpart ULK-2 are the transcriptional targets of tumor suppressor protein p53 in cells. Their transcription is upregulated by p53 during DNA damage which induces autophagy in cells. Similarly, DNA-damaging agents etoposide and camptothecin induce autophagy through this mechanism triggering cell death (Gao et al. 2011). Furthermore, treatment of human colon cancer cell lines with different p53 status including HCT116 (p53 wild-type cells), HCT116/p53KO (p53 knockout cells), RKO and RKO-E6 (p53-blunted), and human bone osteosarcoma epithelial cells (wild type or knock out p53) with DNA-damaging agent camptothecin shows reduced expression of ULK-1 in p53 null cells as compared to p53-positive cells. Additionally, ectopic expression of ULK-1 enhanced autophagy in U2OS cells and shows additive effect with rapamycin on autophagic cell death. Additionally, ULK1 knockdown attenuates ectopically expressed p53-mediated autophagy and cytotoxicity in these cells (Gao et al. 2011). However, the role of ULK-1 in cancer is controversial, and the linearity of its expression with disease prognosis varies in different tumor types. In some tumors, the high expression of ULK-1 is associated with the severity of the disease and overall survival time in patients (Jiang et al. 2011). ULK-1 protein levels are upregulated in esophageal squamous cell carcinoma (ESCC) cell lines and tumor samples as compared to normal esophageal cells and tissues. ESCC cell lines EC109, KYSE140, KYSE510, and KYSE520 have high ULK-1 expression as compared to normal esophageal cell line NE1 (Jiang et al. 2011). Tumor to normal ratio of ULK-1 mRNA isolated from ECACC patients and normal persons is approximately 0.68–1.44-fold high signifying the correlation of ULK-1 with cancer progression. Additionally, the upregulated expression of ULK-1 is inversely correlated with the overall less survival time in patients. Silencing of ULK-1 by gene-specific mRNA induces cytotoxicity and triggers apoptosis in ESCC cell lines (Jiang et al. 2011). These results further point out the protective role of autophagy in cancer cells and the response of tumors against cancer therapy. The upregulation of ULK-1 expression is also found in hepatocellular carcinoma (HCC) patients, and it is significantly associated with tumor size and progression. Patients with low ULK-1 expression have longer survival time than those with high ULK-1 expression (Xu et al. 2013). One of the reasons behind increase in transcription of ULK-1 in solid tumors is the creation of hypoxia. Exposure of cells to hypoxic conditions induces unfolded protein response (UFR) and HIF-1 activation that triggers ULK-1 mRNA transcription (Schaaf et al. 2013). UFR or ER stress activates ATF4 or activating transcription factor 4 which directly binds to the promoter region of ULK-1 DNA and increases its

transcription (Pike et al. 2013). Upregulated ULK-1 induces autophagy which promotes growth and survival of tumor cells during hypoxia. Ablation of ULK-1 or ATF4 in epidermoid carcinoma cell line A431 and breast cancer cell line MCF-7 suppresses autophagy and reduces clonogenic survival of cells, decreases cellular ATP levels, increases cell apoptosis, and reduces spheroid growth (Pike et al. 2013). Loss of function of autophagy protein, FIP200, has been found in many cancers. FIP200 is involved in various cellular process including cell survival, cell growth, cell proliferation, embryonic development, metastasis, and differentiation (Gan and Guan 2008). FIP200 is located on 8q11 chromosome started from 53,535,016 bp from pter to 53,658,403 bp from pter and comprises of 123,388 bases. This region contains several loci of presumptive tumor suppressor genes, and heterozygosity of this region has been linked with various tumor types. Loss of function of this region is present in prostate cancer (Perinchery et al. 1999), breast cancer (Dahiya et al. 1998), colorectal cancer (Staub et al. 2006), hepatocellular carcinoma (Katoh et al. 2005), and ovarian cancer (Dimova et al. 2009). FIP200 deletion in mammary epithelial cells suppresses breast cancer initiation, progression, and metastasis (Wei et al. 2011). Conditional knockout of FIP200 gene *in* mouse model of breast cancer decreases tumor burden and increases overall survival time as compared to control mice containing functional FIP200 gene (Wei et al. 2011). Furthermore, these mice show fewer metastatic nodules as compared to control mice. Deletion of FIP200 causes autophagy defects in MMTV-PyMT transgenic mice (conditional knockout mice or CKO) like accumulation of large ubiquitin-positive or p62-positive aggregates, deformed mitochondria, and deficient LC3 accumulation. These mice show reduced cell proliferation, cell cycle arrest, decreased anchorage-independent growth in soft agar, and glycolysis as compared to control mice. Additionally, FIP200 deletion in Ras-transformed primary mouse embryonic fibroblasts inhibits their proliferation, cell cycle progression, glucose uptake, lactate formation, and anchorage-independent growth in soft agar (Wei et al. 2011). These tumors also show defective autophagy and increased expression of several chemokines including CXCL9 and CXCL10 that initiates increased immune surveillance (Wei et al. 2011). Downregulation of FIP200 by using small interfering RNA triggers apoptotic induction in human glioblastoma cells, immortalized human astrocytes, and primary human brain MvEC. FIP200 directly interacts and inhibits proline-rich tyrosine kinase 2 (Pyk2) expression in these cells and abrogates Pyk2-mediated regulation of calcium ion channels and activation of MEK-ERK signaling (Wang et al. 2011). Another important tumor promoter autophagy gene is lysosomal-associated membrane protein 1 or LAMP-1. It is located on the surface of lysosomes and endosomes and assists lysosomal and autophagosome fusion (Eskelinen 2006). About one third of ovarian serous adenocarcinoma tumors show LAMP-1 over expression in their cytoplasm. Expression analysis of LAMP-1 protein in normal ovarian tissue and ovarian adenocarcinomas of stages IIb, III, and IV reveals high expression of LAMP-1 as compared to normal ovarian tissue (Marzinke et al. 2013). Immunohistochemistry of these tumor lysates shows the presence of LAMP-1 in epithelial cell cytoplasm and few on the surface of plasma membrane. Furthermore, about 73 percent of LAMP-positive adenocarcinomas are positively stained for epidermal growth factor receptor (EFGR) and exhibit moderate to strong EGFR