Chapter 2
Autophagy in Cancer Cells vs. Cancer Tissues: Two Different Stories

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Abstract Autophagy has been considered strongly associated with cancer development and possibly playing important roles in cancer progression. Here we present a computational study of transcriptomic data of cancer tissues, totaling 6317 tissue samples of 11 cancer types along with tissues of inflammatory diseases and cell line based experiments for comparative purposes. Our study clearly revealed that some widely held beliefs and speculations regarding autophagy in cancer may not be well founded, knowing that many of the previous observations were made on cancer cells cultured in man-made environments rather than actual cancer tissues. Our major findings include: (i) the widely used assumption that cancer tissue cells are nutrient depleted is not supported by our tissue-based gene-expression data analysis; (ii) the 11 cancer types studied fall into 2 distinct groups: those with low macro-autophagy (LM) activities and those with high lysosome (HL) activities but induced by

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J.-M. Yang (ed.), *Targeting Autophagy in Cancer Therapy*,
Current Cancer Research, DOI 10.1007/978-3-319-42740-9_2

micro-autophagy and chaperon-mediated autophagy; (ii) co-reduction in autophagy and apoptosis are widely observed in cancer tissues; (iii) down-regulated autophagy strongly correlates with up-regulated cell-cycle progression genes across all cancer types, with one possible functional link detected that repressed autophagosome formation may reduce the degradation of cellular organelles that are essential to cytokinesis, hence contributing to cell cycle progression; (iv) significant correlation is observed between autophagy and immune activities; (v) the down-regulated macroautophagy genes negatively correlate with the total mutation rates in cancer genomes in LM cancers; and (vi) conditional correlation analyses point to a very unexpected direction: cellular Fenton reactions may be the cause of the decreased macroautophagy and its co-expression with apoptosis, increased cell proliferation, genomic mutation rate and even possibly immune response. The information derived here may shed new light on elucidation of fundamental relationships between cancer and autophagy as well as on how to take advantage of the derived relationship for improved treatment of cancer.

2.1 Examining Autophagy in Cancer via Cancer Cell Line *Versus* Cancer Tissues

Autophagy is a cellular survival process under nutrient deprivation and metabolic stress. It degrades cellular proteins, other macromolecules, organelles and cytoplasm; and recycles the nutritious elements to support cell survival. Basal autophagy is a constitutive process that plays a homeostatic function, acting in parallel with the ubiquitin-directed proteasome degradation pathway to maintain the integrity of cellular proteins and organelles. In terms of its role in cancer, the current understanding is: autophagy has a role in supporting cancer cell survival under metabolic stress and in hypoxic regions (Degenhardt et al. 2006). Interestingly, a few essential autophagy genes are found to have high mutation rates across a few types of cancers. For instance, allelic loss of beclin1 gene (BECN1, also known as ATG6) is reported to be among commonly mutated genes in breast, ovarian and prostate cancers (Liang et al. 1999), suggesting that these cancers try to avoid autophagy.

A widely accepted model is that autophagy plays a major survival role throughout cancer initiation and early-stage development by helping cells overcome nutrient deprivation and metabolic stress (Mathew et al. 2007), which are believed to take place in cancer. Its role in more advanced cancers seems to be in stimulation of necrotic cell death, leading to persistent inflammation and repeated wound-healing, an environment that cancer development generally requires (Degenhardt et al. 2006). On the other hand, autophagy is believed to have an important role in maintaining genome integrity by limiting genome damages (Mathew et al. 2007), suggesting that cancers may tend to repress autophagy, knowing that mutations are essential to cancer cell survival. To sort out these complex and conflicting relationships between cancer and autophagy derived through cell line based studies as well as genome

analyses of cancer tissue cells, we need to take a more systematic approach to study a large number of actual cancer tissue samples (*versus* cancer cell lines).

It is noteworthy that there may be fundamental differences between cancer cell line biology and the actual cancer biology. For example, while it has been speculated that cancer cells tend to be low in ATP production based on cell line studies (Lim et al. 2011) (may not be accurate), tissue-based analyses suggest that cancer tissue cells may not be short of ATPs (Gottesman et al. 2002) and our recent study provides an explanation of why this is the case (Hui Yan et al. 2016), suggesting that nutrient deprivation might not necessarily be a valid assumption for autophagy study in cancer tissues.

Here, we present a computational analysis of transcriptomic and genomic data of cancer tissues in the TCGA database (Weinstein et al. 2013), covering 6317 samples of 11 cancer types, aiming to gain a coherent understanding about the relationship between autophagy and cancer. To put our study in a comparative setting, we have also included transcriptomic data of 14 types of inflammatory diseases with 8 being cancer prone and 6 being cancer independent along with control samples plus 12 cell-lined based datasets with experimentally induced autophagy along with controls, to gain a deeper understanding about the actual roles played by autophagy in disease tissues. The detailed information of the omic data used in our study is given in the Sect. 2.9.

The rest of the chapter is organized as follows. Section 2.2 characterizes autophagy in disease tissues and cell lines by comparing transcriptomic profiles of autophagy related genes in both systems. Section 2.3 assesses the level of nutrient deprivation and associated autophagy in cancer and inflammatory disease tissues. Section 2.4 analyzes the interplay between autophagy and apoptosis. Section 2.5 discusses two novel associations between autophagy and cell cycle progression as well as autophagy and immune system. Section 2.6 demonstrates the impact of mutations of autophagy related genes. Section 2.7 discusses about general biological processes that correlate with autophagy. Section 2.8 concludes our prediction of functional roles of autophagy in cancer and inflammatory disease tissues. Section 2.9 covers the methods and data used in this chapter.

2.2 Gene Expression of Autophagy in Disease Tissues

We have conducted differential gene-expression analyses over a total of 6317 disease *versus* control tissue samples of 11 cancer types, 8 cancer-prone inflammatory diseases and 6 cancer-independent inflammatory diseases plus 12 cell-based datasets collected under serum depletion or other metabolic stress conditions for induction of autophagy. An inflammatory disease is considered as *cancer-prone* or *cancer-independent* if the cancer occurrence rate is elevated with statistical significance or not in the disease sites based on published statistics. The details of these diseases and datasets are given in Sect. 2.9. A pathway is considered as *up-* or *down-regulated* if it is enriched with up- or down-regulated genes assessed using a hypergeometric test with p-value = 0.05 as the statistical significance cut-off.

Three types of autophagy have been defined, namely *macro-autophagy*, *micro-autophagy* and *chaperon-mediated autophagy*, which differ in both their induction and execution processes (Glick et al. 2010). To the best of our knowledge, more than 95 % published studies of autophagy in cancer are focused on macro-autophagy (Mizushima 2007).

Eleven autophagy-related pathways are considered in our pathway enrichment analyses, namely: (1) the core gene set of macro-autophagy induction, which consists of the essential genes involved in autophagy initiation, nucleation and expansion; (2) cargo recognition and selectivity genes, covering genes involved in selective autophagy with a recognition function; (3) cytoplasm-to-vacuole targeting pathway (CVT), which is similar to the bulk autophagy (Scott et al. 1996) except that it is activated constitutively under normal growth conditions; (4) autophagy-induction genes, consisting of early response genes that can lead to the induction of autophagy; (5) nucleation assembly genes for the formation of an autophagosome complex; (6) vesicle formation and autophagosome breakdown genes; (7) micro-autophagy invagination pathway (Li et al. 2012); (8) chaperone mediated autophagy genes; (9) lysosome genes; (10) lysosome degradation pathways; and (11) proteasome genes for protein degradation in an autophagy independent manner. The pathway enrichment results in all the aforementioned disease types are listed in Table 2.1.

Our finding is quite surprising as the analysis clearly shows that the expression patterns of the 11 autophagy pathways are substantially different, specifically the macro-autophagy pathways (i.e., the first 6 pathways above) in cancer cell lines *versus* cancer tissues. Specifically, the macro-autophagy and lysosome related pathways are consistently up-regulated in cell-line under nutrient deprivation conditions. However, the macro-autophagy pathways, especially the autophagosome formation genes, including MAP1LC3A, MAP1LC3B, MAP1LC3C, WIPI1, BNIP1, GABARAP, GABARAPL1, GABARAPL2, PARK2, SRPX, LRRK2, ULK2, FYCO1, and TP53INP2, are consistently down-regulated in 7 out of the 11 cancer types, namely THCA, BRCA, HNSC, BLCA, COAD, LUAD and LUSC; and the lysosome pathway is also down-regulated in COAD, LUAD and LUSC. The macro-autophagy recognition, induction and fusion pathways are up-regulated in KICH and STAD; and nucleation assembly pathways are up-regulated in KIRC. Interestingly, KICH, STAD, LICH and BRCA have their micro-autophagy related genes up-regulated; and STAD has its chaperone-mediated autophagy genes up-regulated while none of them are up-regulated in the aforementioned cell-line datasets. Cancer types with up-regulation of at least one autophagy type, namely KICH, STAD, KIRC, LICH and BRCA, all have up-regulated lysosome activity, hence providing a cross-validation between the two predictions and making the them more trustworthy. Interestingly, proteasome genes are generally more up-regulated in cancer types with down-regulated macro-autophagy, suggesting that they may serve similar purposes and hence are mutually exclusive between macro-autophagy and proteasome based protein degradation. Based on these observations, we classify the 11 cancer types into 2 groups: those with low macro-autophagy (LM): BRCA, HNSC, BLCA, COAD, LUAD and LUSC and those with high lysosome (HL): KICH, STAD, KIRC, LIHC and THCA. It is noteworthy that the ATG genes, key autophagy genes, are generally un-differentially expressed across all cancer types.

Table 2.1 Differential expressions of autophagy related pathways across different cancer cells, cancer tissues of different types, and disease tissues of different chronic inflammatory diseases, where cell line represent the overall statistics of the 12 cell-line data, CII and CPI are for cancer-independent and cancer-prone chronic inflammatory diseases, respectively

	Cell line	Cancer											Inflammatory diseases			
	Metabolic stress	KICH	STAD	KIRC	LIHC	THCA	BRCA	HNSC	BLCA	COAD	LUAD	LUSC	CII: PSO, AH, IBS	CII: AST,CS, NAS	CPI: COPD,HCV, CIR, IFP	CPI: UC,CD, BE,AD
Cargo recognition and selectivity	Up						Down			Down	Down	Down	Up			Down
Core	Up						Down	Down	Down	Down	Down	Down	Up			Down
CVT pathway	Up	Up	Up				Down	Down	Down	Down	Down	Down				Down
Induction and fusion	Up	Up	Up							Down			Up			Down
Nucleation assembly	Up			Up		Down	Down	Down	Down	Down	Down	Down	Up			Down
Autophagosomal structure	Up					Down	Down	Down	Down	Down	Down	Down				
Micro-autophagy invagination		Up	Up		Up		Up						Up			
Chaperone mediated autophagy				Up												
Lysosome	Up	Up	Up	Up	Up	Up	Up			Down	Down	Down	Up	Down	Up	Down
Lysosome degradation	Up	Up	Up	Up		Up				Down	Down	Down	Up	Down	Up	Down
Proteasome							Up	Up	Up	Up	Up	Up				

Detailed information of the data is given in Sect. 2.9

Down-regulated macro-autophagy and lysosome are also observed in four types of cancer-prone inflammatory diseases, namely UC, CD, BE and AD while up-regulated lysosome is observed in the other four types of cancer-prone inflammatory diseases: COPD, IFP, HCV and CIR. In comparison, up-regulated macro-autophagy and lysosome pathways are observed in three cancer-independent diseases: PSO, AH and IBS; and down-regulated lysosome is found in the other three cancer-independent diseases: AST, CS and NAS. These strongly suggest that there are no intrinsic relations between cancer and autophagy.

In the remaining portion of the chapter, we focus on elucidation of the possible reasons and functional roles of down-regulation of macro-autophagy and up-regulation of lysosome in LM and HL diseases, respectively.

2.3 Nutrient Deprivation Is Unlikely in Cancer Tissue

It has been repeatedly observed that macro-autophagy can be induced in cancer cells by nutrient deprivation (Mizushima et al. 2004). Hence it has been naturally assumed that cancer tissue cells are also nutrient depleted based on such cell-line studies coupled with observations that cancer tissue cells tend to have substantially increased uptake of glucose (Adekola et al. 2012). However no experimental studies have reliably established that cancer tissue cells are indeed nutrient depleted, to the best of our knowledge. Actually, recent metabolomic studies of cancer tissues suggest that the opposite may be true, i.e., cancer tissue cells are rich in nutrients and in ATPs (Coller 2014). Our recent research provides a possible explanation of where the plentiful ATPs may come from in cancer tissue cells (Hui Yan et al. 2016).

We have recently conducted a modeling analysis based on gene-expression data of 6600+ tissue samples of 14 types of cancer *versus* controls, aiming to assess if there may be Fenton reactions in mitochondria of cancer tissue cells, as strongly suggested by various hints (Hui Yan et al. 2016). Fenton reaction: $Fe^{2+} + H_2O_2 \rightarrow Fe^{3+} + OH^- + \bullet OH$, a non-enzymatic reaction, can take place when the concentrations of Fe^{2+} and H_2O_2 are sufficiently high, which is generally true for chronic inflammatory sites (Winterbourn 1995). The products of the reaction are Fe^{3+}, OH^- and $\bullet OH$. When there are also plentiful reducing elements at or near the reaction sites such as Vitamin C, sulfur or NADH, Fe^{3+} can be reduced to Fe^{2+}, which will enable the reaction to continue. We have developed a computational method to demonstrate if a specific subcellular component may have elevated Fenton reactions or not (Hui Yan et al. 2016), with its basic idea summarized below.

We can rewrite (continuous) Fenton reactions as: $RA + H_2O_2 \rightarrow OH^- + \bullet OH + X$ with Fe^{2+} as the catalyst since Fe^{2+} is not consumed by the (continuous) reaction, with RA and X representing the reducing element and its oxidized form, respectively. We have identified marker genes for each of the five relevant quantities: [RA], [H_2O_2], [OH^-], [$\bullet OH$] and [X] in three cellular compartments: cytosol, mitochondria, and extracellular region, whose expression levels reflect these quantities. We have shown that in tissues without Fenton reactions, these five quantities (for each cellular compartment) are largely independent of each other; and in tissues

having Fenton reactions, they are strongly correlated with each other as measured via the Michaelis-Menton equation (Berg et al. 2002). Using this analysis tool, we have demonstrated that all cancer tissues we studied have Fenton reactions in the three cellular compartments with high statistical significance. Furthermore, we have demonstrated that the OH^- molecules continuously produced by the reaction in mitochondria will lead to reduced concentration of mitochondrial protons, hence leading to a proton gradient on the two sides of the inner membrane of mitochondria, as well as proton influx via the ATP synthase and ATP production just like in a respiration process. The difference is that there is no need for NADHs to push their electrons through the electron transport chain to produce a proton gradient. In sum, when mitochondrial Fenton reactions continue, they will produce ATPs just like in respiration but it does not consume NADHs instead it consumes some reducing elements such as Vitamin C or sulfur.

Using this modeling approach, we also discovered that LM cancers generally have higher levels of cytosolic and extracellular Fenton reactions in comparison with the HL cancers, which tend to have higher mitochondrial but less increased cytosolic Fenton reactions. Knowing that damaged mitochondria can be engulfed by autophagosome and then degraded by lysosome, we posit that up-regulated lysosome-degradation pathway may be involved in the removal of the oxidatively damaged mitochondrial components (Zhou et al. 2011). As a comparison, the down-regulated autophagosome-formation genes in the LM cancers (and inflammatory diseases) are mostly over-expressed in cell line data. All these revealed that the differentially expressed autophagy genes in both of the LM and HL cancers are highly different to metabolic stress induced autophagy in cell lines.

We have also examined in cancer tissues and cell line experiments the expression levels of nine sets of metabolic deprivation responsive genes, which are identified through experiments independent of the expression data used here, under the conditions of deprivation of methionine, leucine, glutamine, amino acids, glucose, and serum (see Sect. 2.9), where the level of differential expression for each gene set reflects the deprivation of a specific metabolite. On average, ~85% (p-value < 1e−30) of the relevant marker genes are differentially expressed in the cell lines treated with each such depletion while <15 % (p-value = 0.6) are differentially expressed in cancer tissues, suggesting a big difference in the level of metabolic deprivation between the cell line based experiments and cancer tissues.

All these observations strongly suggest that nutrient depletion-induced macroautophagy is highly unlikely in cancer tissues.

2.4 Autophagy and Apoptosis

Cross-talks between autophagy and apoptosis have long been known and extensively studied (Maiuri et al. 2007); and both are considered as having tumor suppression functions (Su et al. 2013). We present a computational analysis of expressions of genes involved in both autophagy and apoptosis to assess their

co-expression relations with other autophagy and apoptotic genes to elucidate possible relationships between the two processes in cancer.

Our analyses have detected that a number of core macro-autophagy regulatory genes are down-regulated in cancer tissues but up-regulated in cancer cell line data. Similar patterns of down-regulated core apoptotic regulatory genes such as BCL2L1, BAD, BAG1 and BCL2L11 are observed in tissues of most cancer types, but up-regulated in cancer cell lines. Our co-expression analyses observed positive co-expression among the down-regulated autophagy and apoptosis signaling genes and negative co-expression between the autophagy and proteasome genes in LM cancers. Interestingly, most of the autophagy co-expressed apoptosis genes are up-stream signaling genes, proteasome and ubiquitination genes. The extrinsic and intrinsic apoptotic pathways are largely independent to autophagy in cancer tissues.

We have also observed significant under-expression of several regulatory genes involved in both autophagy and apoptosis such as (1) BH3 binding genes (and complex) BECN1, BNIP3, UVRAG, VPS34, and BCL2L1; (2) DAPK genes: DAPK1, DAPK2 and DAPK3; and (3) ATG5, in both LM and HL cancers. Our co-expression analysis revealed that these genes in LM cancers are strongly co-expressed with a number of proteasome genes, whose up-regulation tends to be strongly associated with cytosolic Fenton reactions, hence supporting our hypothesis that Fenton reaction may be a common reason for the differentially expressed apoptosis regulation and down-regulated autophagy as detailed in Discussion.

2.5 Novel Biological Processes Related to Autophagy

Gene co-expression networks are constructed for each disease type under study, including both cancer and inflammatory diseases, to identify novel biological processes that may associate with the observed down-regulation of macro-autophagy in the LM diseases and up-regulation of lysosome in the HL diseases. We have previously developed a Mutual Rank (MR) based method to detect highly co-expressed gene clusters, also referred to as *co-expression modules* in a global gene co-expression network (Zhang et al. 2015) (see Sect. 2.9).

The method first applies a rank based statistic to detect the significant hub genes in a given co-expression network and then identifies the co-expression module surrounding each hub gene, where hub gene is intuitively defined as a gene with substantially more interaction partners than its neighboring genes in the given co-expression network. The method tends to identify strongly co-expressed gene modules, allowing a gene to be part of multiple modules, hence sensitive to identify novel biological processes correlated with specific targets, say autophagy in the current study. Here, we have identified numerous co-expression modules that are significantly enriched by autophagy genes. Functions of non-autophagy genes in each module are functionally analyzed to reveal novel biological processes strongly associated with autophagy in each disease class. In addition, we

Table 2.2 Information of the data analyzed in this chapter

Cancer type	Cancer label	#Tumor	#Control	Data source	Analyzed mutations
Bladder urothelial carcinoma	BLCA	408	19	TCGA	TP53, PIK3CA, ARID1A, MUC17, MUC16, TTN
Breast invasive carcinoma	BRCA	1095	113	TCGA	TP53, PIK3CA, PTEN, MUC17, MUC16, TTN, MUC4
Colon adenocarcinoma	COAD	285	41	TCGA	TP53, TTN
Head and neck squamous cell carcinoma	HNSC	520	44	TCGA	TP53, PIK3CA, NAV3, MUC17, MUC16, TTN, MUC4
Kidney chromophobe	KICH	66	25	TCGA	TP53
Kidney renal clear cell carcinoma	KIRC	533	72	TCGA	ARID1A, PTEN, NAV3, MUC17, MUC16, TTN
Liver hepatocellular carcinoma	LIHC	371	50	TCGA	TP53, MUC16, TTN
Lung adenocarcinoma	LUAD	515	59	TCGA	TP53, PIK3CA, NAV3, KRAS, MUC17, MUC16, TTN, MUC4
Lung squamous cell carcinoma	LUSC	501	51	TCGA	TP53, PIK3CA, NAV3, MUC17, MUC16, TTN, MUC4
Stomach adenocarcinoma	STAD	238	33	TCGA	TP53, PIK3CA, ARID1A, PTEN, NAV3, KRAS, MUC17, MUC16, TTN, MUC4
Thyroid carcinoma	THCA	505	59	TCGA	

Inflammatory disease types	Disease label	#Disease	#Control	Data source	Relevance to cancer
Crohn's disease	CD	37	12	GSE16879	Risking
Ulcerative colitis	UC	40	12	GSE16879	Risking
Liver cirrhosis	CIR	13	10	GSE6764	Risking
Barrett's esophagus	BE	20	19	GSE26886	Risking
HCV infection	HCV	16	2	GSE11190	Risking
Chronic obstructive pulmonary disease	COPD	35	63	GSE11784	Risking
Idiopathic pulmonary fibrosis	IFP	23	6	GSE21369	Risking
Atopic dermatitis	AD	13	8	GSE32924	Risking
Psoriasis	PSO	33	21	GSE14905	Independent
Asthmatics	AST	42	28	GSE4302	Independent
Alcohol hepatitis	AH	15	7	GSE28619	Independent
Non-alcoholic steatohepatitis	NAS	9	7	GSE63067	Independent

(continued)

Table 2.2 (continued)

Inflammatory disease types	Disease label	#Disease	#Control	Data source	Relevance to cancer
Irritable bowel syndrome	IBS	28	77	GSE36701	Independent
Cutaneous sarcoidosis	CS	15	5	GSE32887	Independent

Cell line experiments	Cell type	#Treated	#Control	Data source
Serum deprivation	T98G cell	3	3	GSE1692
Glucose deprivation	MCF7 cell	5	6	GSE19123
Starvation-induced autophagy	Lymphoblastoid cell line	3	3	GSE2435
Serum starvation	Lymphoblastoid cell line	6	6	GSE31040
Glucose deprivation	HCT116 cell	9	9	GSE38061
Tunicamycin treatment	PC3 cell	1	1	GSE38643
Induction of autophagy by atorvastatin	PC3 cell	2	2	GSE46376
GANT61 treatment	ES2 and H4	2	2	GSE54936
Glucose deprivation	A549 cell	2	2	GSE56843
4-hydroxytamoxifen treatment	IMR90 cell	6	6	GSE59522
Serum deprivation	LoVo cell	3	3	GSE70976
Serum deprivation	T cell	3	10	GSE7497

have also conducted a similar analysis but on co-expression modules enriched by lysosome genes. These two classes of modules are referred to as autophagy- and lysosome-centric modules in the following. Table 2.2 lists all such modules, along with their annotated functions for each disease type under study.

2.5.1 Autophagy and Cell Cycle Control

We noted that cell cycle genes, specifically the G2-M transition genes, enrich at least 30 % of the autophagy-centric co-expression modules in each LM cancer type while G1-S transition genes and other cell cycle genes enrich ~20 % of the lysosome-centric modules in HL cancers. Further analysis revealed that cell-cycle genes are negatively correlated with the down-regulated autophagy genes in LM cancers and positively correlated with the up-regulated lysosome genes in HL cancers. Figure 2.1 shows the co-expression networks among autophagy and cell cycle genes identified in each cancer type.

Macro-autophagy may suppress cell-cycle progression through blocking the transition from G2 to M (Kuo et al. 2011; Matsui et al. 2013). A recent study suggests that autophagy may also have important roles in suppression of cytokinesis (Kuo et al. 2011). Our analysis detected a consistent negative correlation between

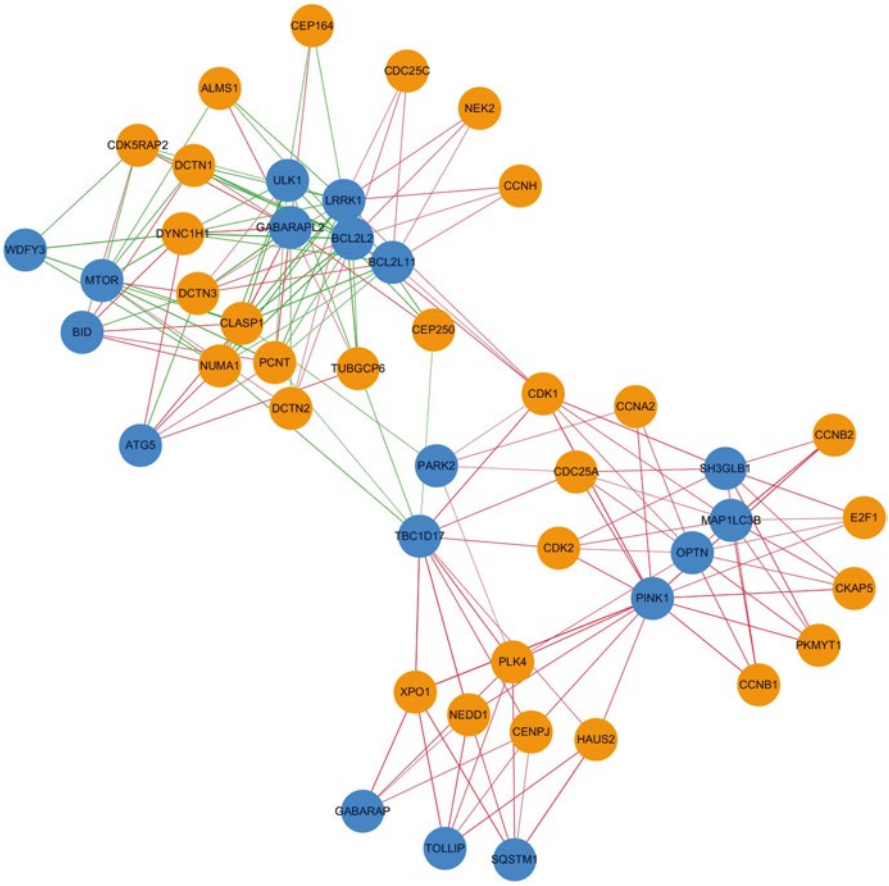

Fig. 2.1 Co-expression network among down-regulated autophagy genes and up-regulated cell cycle genes in COAD. The autophagy genes are represented by *blue* nodes and the cell cycle genes are in *orange*. The *red* and *green edges* represent negative and positive co-expression, respectively, and the width of the edge denotes the level of co-expression

the over-expressed cyclins CCNB1 and CCNB2, cyclin dependent kinases CDK1 and CDK2, a number of centrosomal protein genes, other G2-M transition genes *versus* all the under-expressed macro-autophagy genes: ATG5, ATG7, ATG10, ATG12, GABARAP, GABARAPL2, MAP1LC3B, and PARK2, all related to formation and maturation of autophagosome in the following LM cancers: COAD, LUAD and LUSC. With the knowledge that autophagic degradation may be involved in the cleaning of midbody derivatives after cytokinesis (Pohl and Jentsch 2009; Kuo et al. 2011), we speculate that suppression of the autophagosome formation may preserve the organelles that are necessary for cell proliferation in cancer tissues.

Further analysis revealed that most of the up-regulated G1-S transition genes in HL cancers are highly co-expressed with the over-expressed lysosome genes are proteasome genes. The cyclins, cyclin dependent kinases, DNA polymerases and other cell-cycle regulatory genes are largely independent of the lysosome genes. We speculate that the co-expression between lysosome and proteasome just reflect a normal relationship between the two protein degradation systems, which are both up-regulated in cancer probably due to the increased damage to proteins possibly by ROS (Waris and Ahsan 2006).

2.5.2 *Autophagy and Immune Response*

Our analysis has identified that the down-regulated genes involved in autophagosome formation and maturation, such as MAP1LC3C, SNCA, ATG7, ATG4C, ATG12, ATG5, OPTN, GABARAP, PARK2, and SH3GLB1 are significantly

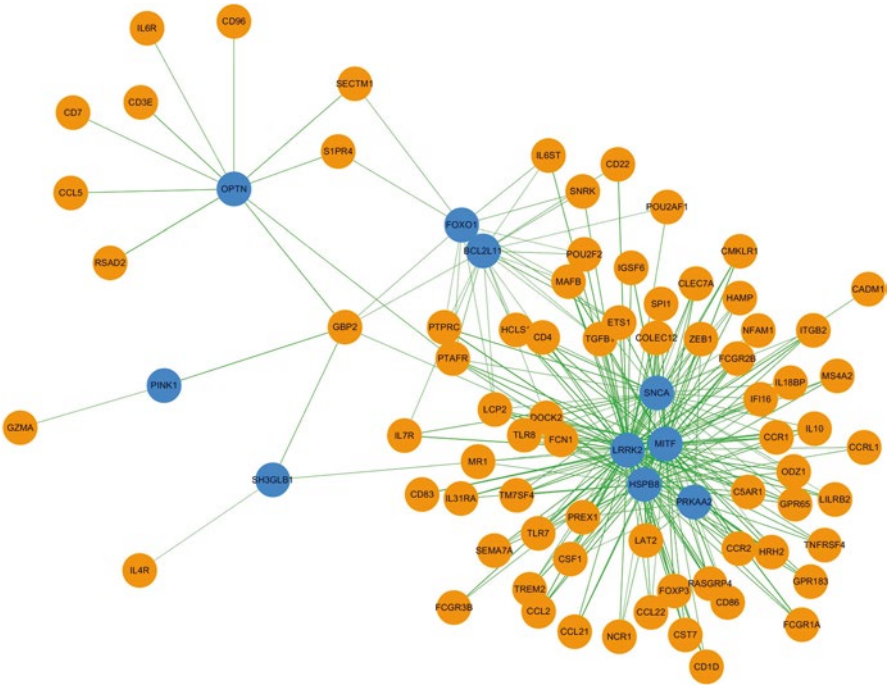

Fig. 2.2 Co-expression network among autophagy and immune response genes in COAD. The autophagy genes are represented by *blue* nodes and the immune response genes by *orange* nodes. The *green* edges represent positive co-expressions and the width of an edge denotes the level of co-expression

co-expressed with 81 under-expressed immune response genes in LM cancers. Similarly, significant co-expression is observed between the down-regulated autophagy genes and immune response genes in LM (and cancer prone) inflammatory diseases. In comparison, 57 up-regulated lysosome genes are strongly co-expressed with 178 up-regulated immune response genes in HL cancers. Interestingly, immune response genes that are co-expressed with autophagy genes have substantial overlap between LM and HL cancers but with opposite differential expression patterns, i.e., down vs. up-regulation. These immune response genes include CD markers, chemokine ligands, chemokine receptors, interleukins, interleukin receptors and other immune genes. The co-expression modules consisting of both autophagy and immune response genes are shown in Fig. 2.2 for selected diseases.

From Fig. 2.2, we can see that these co-expression modules contain a large number of genes related to multiple immune cell types such as CD4+ and CD8+ T cells, B cells, dendritic cells, natural killer cells and macrophages. This is consistent with the observation that the co-expression modules enriched with immune response and autophagy genes are also substantially enriched by lipid binding, lipid metabolism and glycosaminoglycan metabolic genes as it is known that increased lipid metabolism tends to trigger increased immune response (Fritsche 2006) and the same with increased synthesis of cell-surface glycan (Zhang 2006).

Previous studies have identified various invading microbes such as HBV and H. Pylori have developed ways to evade autophagy by suppressing autophagosome formation and fusion to lysosome (Tang et al. 2012), which is consistent with what we observed in the LM cancers and cancer-prone inflammation. The autophagy evasion mechanism has been observed in host cells causing a failed degradation of the infected cells hence less antigen presenting (Paludan et al. 2005). We speculate that normal autophagy in cancer tissue can degrade damaged macromolecules and organelles to promote immune response through induction of antigen presenting. Such a mechanism can be hindered in LM cancers by the suppressed autophagosome formation that is possibly caused by extracellular and cytosolic Fenton reaction, as discussed in Discussion.

2.6 Autophagy and Genomic Mutation

We have conducted a comparative analysis between the level of autophagy and genomic mutation rate using cancer genomic sequences and matching transcriptomic data in TCGA, to assess correlations between genomic mutations and the expression levels of autophagy genes.

We have computed correlation between the somatic mutation rate and gene expression level of autophagy-related genes for each cancer type to evaluate if there is any correlation between the level of differentiation of autophagy genes and the mutation rate at the whole genome level for each cancer type under

study. Interestingly, while correlations are detected, they are different for different cancer types. We noted: the expression levels of lysosome genes are generally positively correlated with the mutation rate in KIRC, KICH and STAD cancers while some sets of macro-autophagy genes are either positively or negatively correlated with the mutation rate in COAD, LUAD, BLCA, and BRCA with strong statistical significance. This correlation is insignificant in LICH, THCA, LUSC, and HNSC.

Genes involved in autophagosome formation such as ATG2B, TP53INP2, SQSTM1, FYCO1, GABARAPL1, GABARAPL2, GABARAPL3, MAP1LC3A, MAP1LC3B and MAP1LC3C are negatively correlated with the mutation rates. They are under-expressed in 8 out of the 11 cancer types, covering all LM cancers. We noted that a number of pro-autophagy and pro-apoptosis signaling genes such as ATG16L1, SKP2, BAX, BID, RPS6KB1, and PIK3R2 are positively correlated with the mutation rate; and they are up-regulated in eight cancer types. Seven autophagy core signaling genes: ATG3, ATG4A, ATG4C, ATG4D, ATG5, ATG7, and ATG12 are positively correlated with the mutation rate but they are not significantly differentially expressed. In addition, the proteasome genes are generally up-regulated and positively correlated with the mutation rate in LM cancers. One possible explanation is that increased proteasome activities tend to be associated with increased Fenton reactions, which can lead to increased mutations, as further discussed in Sect. 2.7.

We have also examined correlations between expressions of autophagy genes and non-synonymous mutation of six specific cancer gene mutations: TP53, KRAS, NAV3, PTEN, ARID1A, and PIK3CA and four frequently mutated genes namely MUC4, TTN, MUC16, and MUC17. TP53 mutation rate is highly positively correlated with the expression levels of a large number of autophagy signaling genes such as ATG genes and BCL-2 genes in BRCA and COAD. WIPI2, RB1CC1 and ATG9A, involved in regulation of the autophagosome formation, are down-regulated in BRCA, COAD and STAD tissues harboring PI3KCA mutations, which are known to affect autophagy responses through the PI3K pathway (Shanware et al. 2013). No significant association has been observed between the expressions of autophagy genes and the other examined gene mutations.

2.7 Discussion

The role of autophagy in cancer has long been debated as it is proposed to promote cancer cells' survival under certain stresses and also possibly to have tumor-suppression roles (Mathew et al. 2007). These studies have been predominantly conducted on cell lines with induced autophagy, hence naturally raising a question: are such observations applicable to cancer tissues, knowing

that the environments in cancer tissues could be substantially different from cell line studies?

We have recently conducted a comparative analysis of gene-expression of cancer tissues and cancer cell lines of the matching cancer types, namely BLCA, BRCA, COAD, KIRC, LIHC, LUAD, LUSC, PRAD, STAD and THCA, under multiple conditions to assess the expression patterns of 896 well-characterized biological pathways, covering 48.83 % (10,010/20,501) of human genes in the two systems (Wei Du and Xu 2016). The following interesting and informative observations are made: (1) 83.15 % of the pathways are found to have similar expression patterns under multiple conditions across different cell lines for the same cancer type; (2) 96.98 % of the pathways share similar expression patterns across different gene-expression datasets for the same cancer type; and (3) only 20.42 % of the pathways share similar expression patterns between cancer tissue samples and cell line datasets. This clearly raised a legitimate concern regarding the applicability of cell-line based observations, particularly about autophagy since our study has clearly shown that there are fundamental differences between autophagy in the two systems.

From the comparative analyses presented throughout this chapter, we noted that the following main differences between cancer tissues and cancer cell based studies:

- The 11 cancer types and 8 cancer prone inflammatory diseases studied here all have reduced or unchanged macro-autophagy activities compared to their basal level activities in the control samples, which is opposite to what has been observed in cancer cell lines under induced metabolic stresses;
- The 11 cancer types full into 2 classes, 1 with reduced macro-autophagy activities and the other with elevated lysosome activities associated with increased micro-autophagy or chaperon-mediated autophagy; reduced macro-autophagy is also observed in 4 cancer prone chronic inflammatory diseases;
- The differentially expressed autophagy genes are largely independent of the enzymes involved in energy metabolism across all the examined disease tissues. Furthermore, there does not seem to be any nutrient-deprivation induced macro-autophagy in cancer tissues;
- Co-expression between altered autophagy activity levels and each of the following: apoptosis, genomic mutation rate and to some level immune response seems to have a strong influence from Fenton reactions in cancer tissues, which cancer cells generally do not have.

Through co-expression analyses, we have identified numerous non-autophagy genes that are strongly co-expressed with various autophagy genes. Out of these genes, some could be the direct causes or results while others may co-occur with the altered autophagy activities, both as results of some common causes. We have conducted further computational analyses to assess if some of these co-expressed genes with autophagy genes may be the result of one specific common cause, Fenton reactions.

As discussed earlier, we consider Fenton reactions as one of the root causes of malignant transformation from normal cells to neoplastic cells. Specifically, we have demonstrated that all cancer tissues have Fenton reactions in cytosol, mitochondria and extracellular space (Hui Yan et al. 2016) and cancer-prone inflammatory tissue cells have the reaction in some but not all three subcellular compartments (Chi Zhang and Xu 2016). By using the Michaelis-Menton equation, we have developed a computational procedure to assess if a specific subcellular location may have Fenton reaction via estimating the four relevant quantities modeled by Michaelis-Mention equation (see below) using gene-expression data. Specifically, the following equation represents the model for estimating the quantity of OH produced by Fenton reaction in a specific subcellular location:

$$[\cdot OH] = \sum_{XH_2} \frac{K_{cat}^{RA}\left(\sum_i a_i X_i^{Fe} + a_0\right)}{\frac{K_1^{XH_2}}{1} + \frac{K_2^{RA}}{\sum_j b_j X_j^{ROS} + b_0} + \frac{K_3^{RA}}{\sum_k c_k X_k^{RA} + c_0} + \frac{K_4^{RA}}{\left(\sum_j b_j X_j^{ROS} + b_0\right)\left(\sum_k c_k X_k^{RA} + c_0\right)}} + \varepsilon,$$

where X_i^{Fe}, X_j^{ROS}, and X_k^{RA} denote the gene expression of marker genes to estimate the level of Fe^{2+}, H_2O_2 and RA by the following linear models:

$$\left[Fe^{2+}\right] = \sum_i a_i X_i^{Fe} + a_0 + \varepsilon_{Fe}$$

$$\left[H_2O_2\right] = \sum_j b_j X_j^{ROS} + b_0 + \varepsilon_{ROS}$$

$$[RA] = \sum_k c_k X_k^{RA} + c_0 + \varepsilon_{RA},$$

where a_i, b_i, and c_i are regression parameters and ε, ε_{Fe}, ε_{ROS}, *and* ε_{RA} are errors.

We have checked if the observed significant co-expressions between autophagy and other biological processes: cell cycle process, mutation rate, and immune response, are possibly causally linked with each other or are common results of Fenton reactions. Statistically, we have checked the co-expression level between A (autophagy) and B (one of the other biological processes), denoted as cor(A, B), and compared this with the same co-expression level but under condition of C (Fenton reaction), denoted as cor(A, B | C). The contribution of C to the co-expression between A and B is evaluated by the *Ratio of Significant Conditional Dependence* (RSCD) defined as the ratio of the number of significantly correlated gene pairs from A and B *versus* the number of significantly correlated gene pairs from A and B under condition of C (see

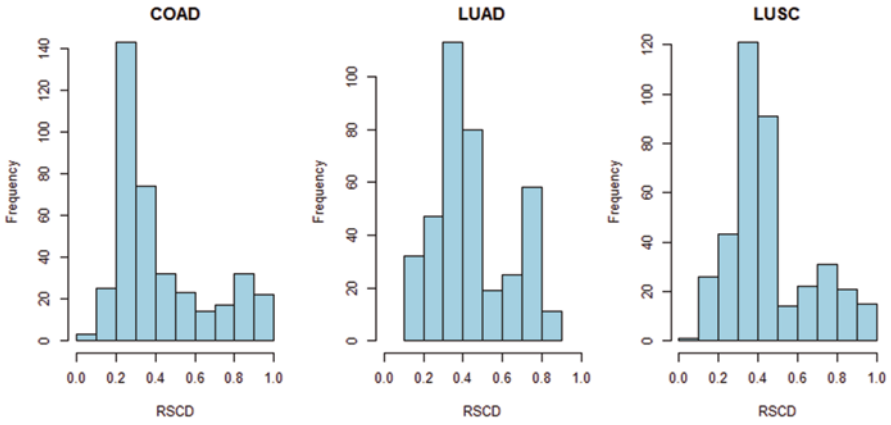

Fig. 2.3 Distribution of the RSCD (A,B|C) values with A = down-regulated autophagosome formation genes, B = the top 400 biological processes co-expressed with the autophagosome formation genes, and C = cytosolic Fenton reaction in three LM cancer types

Sect. 2.9). Smaller RSCD values indicate higher impact of C on the correlation between A and B.

We have computed the RSCD values for A = down-regulated autophagosome formation genes, B = the top 400 biological processes co-expressed with A, and C = cytosolic Fenton reaction in the LM cancer types. Histograms of the RSCD values for COAD, LUAD and LUSC are plotted in Fig. 2.3. From the figure, we can see that the RSCD values in the three cancer types are consistently distributed as bimodal distributions with one large peak for low RSCD values (<0.6) and a small peak for high RSCD (>0.6) values, strongly suggesting most of the biological processes co-expressed with the down-regulation of autophagosome formation genes are dependent on cytosolic Fenton reactions. Further analysis revealed that the biological processes with low RSCD values are quite consistent among the three cancer types, namely apoptosis, cell cycle, DNA binding, ion binding, mitochondria, nucleotide synthesis, Golgi apparatus, mRNA transcription, translation and organelles. It is noteworthy that the centrosome genes have the highest RSCD values among all the proliferation related pathways, substantiating our hypothesis that other than being the common results of cytosolic Fenton reactions, decreased autophagosome formation may directly influence cell cycle process. Considering the biological properties of A, B, and C, we speculate that the best explanation of the observations is that cytosolic Fenton reactions are a common reason for the down-regulated autophagosome formation and other co-expressed biological processes.

Interestingly, most of the immune related genes and pathways do not seem to have influence from cytosolic Fenton reactions as revealed by the above analysis (with high RSCD values). Hence we then conducted a similar analysis but

using extracellular Fenton reactions, and have the following results. The average RSCD value of the immune and inflammation related pathways conditional to cytosolic Fenton reaction is around 0.8 in the three cancer types while the average RSCD value of the pathways conditional to extracellular Fenton reaction is 0.5, suggesting a significant contribution of extracellular Fenton reaction on the correlation between immune response and autophagy but still directly interactions between them exist there. Possible explanations of the observation include (1) the macro-autophagy contribute to cytokine production as in infectious diseases, hence suppressed autophagosome cause less cytokine releasing and immune surveillance (Harris 2011); (2) the macro-autophagy is suppressed by signaling pathways of certain interleukins include IL-4, IL-10 and IL-13 as a result of deregulated immune response (Lapaquette et al. 2015); and (3) extracellular Fenton reaction is a common reason for the immune response and suppressed autophagosome in the LM cancers.

2.8 Conclusion

Our comparative analysis of transcriptomic data of cancer tissues *versus* cancer cell lines revealed that (1) cancer tissues generally do not have metabolic stress and its induced macro-autophagy; (2) while some cancer tissues have increased lysosome activity, it is largely induced micro-autophagy or chaperon-mediated autophagy for degradation of oxidatively damaged macromolecules and organelles due to Fenton reactions; and (3) various cellular processes are found to be co-expressed with autophagy genes; the majority of them may represent co-occurring events with autophagy as common results of Fenton reactions, rather than causal relations with autophagy. Overall, new information is revealed, which is clearly subject to further experimental validation and may possibly lead to improved understanding about the biology of autophagy in cancer tissues.

2.9 Material and Methods

2.9.1 Data Used

We have conducted a differential gene expression analysis measured using normalized fold change in 11 TCGA cancer types: namely bladder urothelial carcinoma (BLCA), breast invasive carcinoma (BRCA), colon adenocarcinoma (COAD), head and neck squamous cell carcinoma (HNSC), kidney chromophobe (KICH), kidney renal clear cell carcinoma (KIRC), liver hepatocellular carcinoma (LIHC), lung adenocarcinoma (LUAD), lung squamous cell carcinoma (LUSC), stomach adenocarcinoma (STAD), and thyroid carcinoma (THCA);

eight cancer-prone inflammatory diseases: cirrhosis (CIR), Barrett's esophagus (BE), ulcerative colitis (UC), Crohn's disease (CD), chronic HCV infection (HCV), idiopathic pulmonary fibrosis (IPF), chronic obstructive pulmonary disease (COPD), and atopic dermatitis (AD) that have significant increased risk for cancer development; and six cancer-independent inflammatory diseases namely alcohol hepatitis (AH), non-alcoholic steatohepatitis (NAS), cystitis (CS), asthmatics (AST), and psoriasis (PSO) whose occurrence does not increase the risk of cancer development as reported in literature; and 12 datasets of cell line-based gene-expression data collected under serum depletion or other metabolic stress conditions to induce autophagy.

RNA-seq and genomic data of these tissue and cell samples are collected from TCGA and GEO databases. Detailed information of the datasets are listed in Table 2.2. The RNA-seq data are normalized using the RSEM method while all the microarray data are measured by UA133 plus 2.0 array and normalized by the RMA method.

2.9.2 Gene Differential Expression and Pathway Enrichment Analysis

Differentially expressed genes in cancer and inflammatory disease are assessed by using the Mann-Whitney test with p-value adjusted by the FDR method and FDR = 0.05 is used as the significance cut-off. Average Fold Change (FC) is used on cell line gene expression under autophagy-inducing condition *versus* controls for determination of differentially expressed genes due to their limited sample size. We use log (FC) = 0.5 and –0.5 as the cut-off for over and under expression, respectively.

Eleven autophagy related pathways are manually generated, as detailed in the main text. 2775 pathways covering major biological processes including cell proliferation, apoptosis, and immune response among a few others are collected from the MsigDB database. In addition, nine sets of metabolic deprivation marker genes responsive to glucose, leucine, methionine, glutamate, other amino acids as well as serum deprivation are also retrieved from the MsigDB chemical perturbation responsive gene sets.

Pathway enrichment is assessed using a hypergeometric test and the p-values are adjusted by the FDR method with FDR = 0.05 as the significance cut-off.

2.9.3 Gene Co-expression Analysis

We have previously developed a Rank-based gene co-expression module extraction method (Zhang et al. 2015). The method first identifies hub genes in a given gene network and then expand the hubs to co-expression modules. The method is applied

to the cancer and inflammatory disease data to identify co-expression modules that are significantly enriched by autophagy genes. The co-expression modules enriched by down-regulated macro-autophagy genes in LM diseases and up-regulated lysosome genes in HL diseases are specifically analyzed to elucidate biological processes related to the differentially expressed autophagy genes.

2.9.4 Correlate Gene Expression Data to Genomic Mutations

We define the total mutation rate of each sample as the total number of non-synonyms point mutations. Pearson correlation coefficient between the expression level of each gene and the mutation rate for each disease type is calculated. Significance of the correlation is assessed by using the *t*-test. Six cancer genes and four frequently mutated genes are selected and analyzed. Association between each mutation and autophagy gene is tested by comparing the gene expression level in sample with the mutation *versus* the mutation free samples by Mann Whitney test. FDR = 0.05 is used as the significance cut-off.

2.9.5 Ratio of Significant Conditional Dependence

The Ratio of Significant Conditional Dependence (RSCD) is defined by the ratio of the number of significantly correlated gene pairs from sets A and B *versus* the number of significantly correlated gene pairs from A and B under condition C. The RSCD is defined by following:

$$\mathrm{RSCD}(\mathrm{A},\mathrm{B}\mid\mathrm{C}) = \frac{\#\left\{\mathrm{p.cor}\left(G_a, G_b\right)\alpha \mid G_a \in A, G_b \in B\right\}}{\#\left\{\mathrm{p.cor}\left(G_a, G_b \mid C\right) < \alpha \mid G_a \in A, G_b \in B\right\}},$$

where G_a and G_b are a pair of genes from pathway A and B, p.cor is the p-value of the co-expression and α is the significance level. We use Pearson correlation to evaluate the co-expression level between gene pairs. The Pearson correlation product-moment function is applied to access the p-value for each correlation (and conditional correlation) and $\alpha = 0.05$ is used as the statistical significance cut-off. There is a significant contribution by C to the correlation between A and B if the conditional correlation level Cor (A, B | C) is substantially lower than Cor (A, B), and there is no significant contribution by C to the correlation between A and B if Cor (A, B | C) is comparable with Cor (A, B). Hence smaller RSCD values imply higher impact of C on the correlation between A and B.

References

Adekola, K., Rosen, S. T., & Shanmugam, M. (2012). Glucose transporters in cancer metabolism. *Current Opinion in Oncology, 24*(6), 650–654.

Berg, J. M., Tymoczko, J. L., & Stryer, L. (2002). *The Michaelis-Menten model accounts for the kinetic properties of many enzymes*. New York: WH Freeman.

Chi Zhang, F. Y., & Xu, Y (2016). Elucidation of fundamental differences between cancer prone and cancer independent chronic inflammatory diseases (in preparation). http://csbl.bmb.uga.edu/~zhangchi/FentonReaction/index.html.

Coller, H. A. (2014). Is cancer a metabolic disease? *The American Journal of Pathology, 184*(1), 4–17.

Degenhardt, K., Mathew, R., Beaudoin, B., Bray, K., Anderson, D., Chen, G., et al. (2006). Autophagy promotes tumor cell survival and restricts necrosis, inflammation, and tumorigenesis. *Cancer Cell, 10*(1), 51–64.

Fritsche, K. (2006). Fatty acids as modulators of the immune response. *Annual Review of Nutrition, 26*, 45–73.

Glick, D., Barth, S., & Macleod, K. F. (2010). Autophagy: Cellular and molecular mechanisms. *The Journal of Pathology, 221*(1), 3–12.

Gottesman, M. M., Foto, T., & Bates, S. E. (2002). Multidrug resistance in cancer: Role of ATP-dependent transporters. *Nature Reviews Cancer, 2*(1), 48–58.

Harris, J. (2011). Autophagy and cytokines. *Cytokine, 56*(2), 140–144.

Hui Yan, C. Z., Dong, N., Sheng, T., & Xu, Y. (2016). Fenton reactions are a fundamental driver of cell division in cancer (under review). http://csbl.bmb.uga.edu/~zhangchi/FentonReaction/index.html.

Kuo, T.-C., Chen, C. T., Baron, D., Onder, T. T., Loewer, S., Almeida, S., et al. (2011). Midbody accumulation through evasion of autophagy contributes to cellular reprogramming and tumorigenicity. *Nature Cell Biology, 13*(10), 1214–1223.

Lapaquette, P., Guzzo, J., Bretillon, L., & Bringer, M. A. (2015). Cellular and molecular connections between autophagy and inflammation. *Mediators of Inflammation, 2015*, 398483.

Li, W.-W., Li, J., & Bao, J. K. (2012). Microautophagy: Lesser-known self-eating. *Cellular and Molecular Life Sciences, 69*(7), 1125–1136.

Liang, X. H., Jackson, S., Seaman, M., Brown, K., Kempkes, B., Hibshoosh, H., et al. (1999). Induction of autophagy and inhibition of tumorigenesis by beclin 1. *Nature, 402*(6762), 672–676.

Lim, H. Y., Ho, Q. S., Low, J., Choolani, M., & Wong, K. P. (2011). Respiratory competent mitochondria in human ovarian and peritoneal cancer. *Mitochondrion, 11*(3), 437–443.

Maiuri, M. C., Zalckvar, E., Kimchi, A., & Kroemer, G. (2007). Self-eating and self-killing: crosstalk between autophagy and apoptosis. *Nature Reviews Molecular Cell Biology, 8*(9), 741–752.

Mathew, R., Karantza-Wadsworth, V., & White, E. (2007). Role of autophagy in cancer. *Nature Reviews Cancer, 7*(12), 961–967.

Mathew, R., Kongara, S., Beaudoin, B., Karp, C. M., Bray, K., Degenhardt, K., et al. (2007). Autophagy suppresses tumor progression by limiting chromosomal instability. *Genes & Development, 21*(11), 1367–1381

Matsui, A., Kamada, Y., & Matsuura, A. (2013). The role of autophagy in genome stability through suppression of abnormal mitosis under starvation. *PLoS Genetics, 9*(1), e1003245.

Mizushima, N. (2007). Autophagy: Process and function. *Genes & Development, 21*(22), 2861–2873.

Mizushima, N., Yamamoto, A., Matsui, M., Yoshimori, T., & Ohsumi, Y. (2004). In vivo analysis of autophagy in response to nutrient starvation using transgenic mice expressing a fluorescent autophagosome marker. *Molecular Biology of the Cell, 15*(3), 1101–1111.

Paludan, C., Schmid, D., Landthaler, M., Vockerodt, M., Kube, D., Tuschl, T., et al. (2005). Endogenous MHC class II processing of a viral nuclear antigen after autophagy. *Science, 307*(5709), 593–596.

Pohl, C., & Jentsch, S. (2009). Midbody ring disposal by autophagy is a post-abscission event of cytokinesis. *Nature Cell Biology, 11*(1), 65–70.

Scott, S. V., Hefner-Gravink, A., Morano, K. A., Noda, T., Ohsumi, Y., & Klionsky, D. J. (1996). Cytoplasm-to-vacuole targeting and autophagy employ the same machinery to deliver proteins to the yeast vacuole. *Proceedings of the National Academy of Sciences, 93*(22), 12304–12308.

Shanware, N. P., Bray, K., & Abraham, R. T. (2013). The PI3K, metabolic, and autophagy networks: Interactive partners in cellular health and disease. *Annual Review of Pharmacology and Toxicology, 53*, 89–106.

Su, M., Mei, Y., Sinha, S. (2013). Role of the crosstalk between autophagy and apoptosis in cancer. *Journal of Oncology 2013*, 14.

Tang, S.-W., Ducroux, A., Jeang, K. T., & Neuveut, C. (2012). Impact of cellular autophagy on viruses: Insights from hepatitis B virus and human retroviruses. *Journal of Biomedical Science, 19*, 92. doi:10.1186/1423-0127-19-92.

Waris, G., & Ahsan, H. (2006). Reactive oxygen species: Role in the development of cancer and various chronic conditions. *Journal of Carcinogenesis, 5*(1), 14.

Wei Du, N. D., & Xu, Y (2016). On fundamental differences between cancer tissue cells and cancer cell lines (in preparation). http://csbl.bmb.uga.edu/~zhangchi/FentonReaction/index.html.

Weinstein, J. N., Collisson, E. A., Mills, G. B., Shaw, K. R., Ozenberger, B. A., Ellrott, K., et al. (2013). The cancer genome atlas pan-cancer analysis project. *Nature Genetics, 45*(10), 1113–1120.

Winterbourn, C. C. (1995). Toxicity of iron and hydrogen peroxide: The Fenton reaction. *Toxicology Letters, 82*, 969–974.

Zhang, X.-L. (2006). Roles of glycans and glycopeptides in immune system and immune-related diseases. *Current Medicinal Chemistry, 13*(10), 1141–1147.

Zhang, C., Liu, C., Cao, S., & Xu, Y. (2015). Elucidation of drivers of high-level production of lactates throughout a cancer development. *Journal of Molecular Cell Biology, 7*(3), 267–279.

Zhou, Q., Li, H., & Xue, D. (2011). Elimination of paternal mitochondria through the lysosomal degradation pathway in C. elegans. *Cell Research, 21*(12), 1662–1669.

http://www.springer.com/978-3-319-42738-6

Targeting Autophagy in Cancer Therapy
Yang, J.-M. (Ed.)
2016, VII, 141 p. 12 illus., 10 illus. in color., Hardcover
ISBN: 978-3-319-42738-6