Role of Autophagy in the Myelodysplastic Syndrome and Myeloproliferative Disorders

Shaimaa F. Kamel[1], Mohamed E. Abd El-Fattah[1], Hisham A. Aly[1], Hisham M. Tawfik[1], Elwy M. Soliman[1], Khaled M. Salah[2]

[1]Internal Medicine Department, [2]Clinical Pathology Department, Faculty of Medicine, Minia University, Egypt

Abstract

Objective: Modulation of autophagy is a promising potential strategy enhancing cancer therapy, especially after development of new autophagy inhibitors. We planned to assess the role of autophagy in Myelodysplastic syndrome (MDS) and Myeloproliferative neoplasms (MPNs).

Design: Case controlled single center study**.**

Setting: Internal medicine department, Minia University Hospital, Minia, Egypt.

Subjects: A total of 70 subjects divided into two groups: group I: 35 newly diagnosed patients with MDS and MPNs and group II: 35 apparently normal individuals.

Intervention: Serum beclin-1 (S.BECN 1) and Serum autophagy protein 7(S. Atg-7) were measured in both groups.

Main Outcome Measure: We considered the following parameters: demographic data, bone marrow examination, splenomegaly, hepatomegaly and laboratory investigations in both patients and control group including beclin1 and Atg7.

Results: By comparingserum beclin1 level in the two groups: patients group showed that themean and SDof both S. beclin land S. Atg-7were much higher than the control group. This shows highly significant P value <0.001*.

Conclusion: Measurement of high level of S.BECN 1 and S. Atg 7 in MDS and MPNs indicate that they may be autophagy-dependent markers and it may be modifiable factors through its inhibition or induction.

Keywords: *Autophagy, Myelodysplastic syndrome; Myeloproliferative neoplasms.*

Introduction

The term of Autophagy was firstly described by Christian de duve in 1963, who coined that autophagy is a "self-eating". He observed that the cell could destroy its own contents by enclosing it in membranes, forming sack-like vesicles that were transported to a recycling compartment, called lysosome, for degradation. In 2016, Nobel Prize in physiology or medicine was awarded to yoshinori oshumi for his discovery the mechanism of autophagy [1].

Autophagy is a cellular pathway responsible for the sequestration of spent organelles and protein aggregates from the cytoplasm and their delivery into lysosomes for degradation[2].Whereby cellular proteins and organelles are engulfed by autophagosomes, digested in lysosomes, and recycled to sustain cellular metabolism [3].

Corresponding Author:
Shaimaa F. Kamel
Internal medicine Department, Minia University, Egypt
e-mail: massa2000@yahoo.com
Tel: 02 01005651501

Autophagy has been shown to play several important roles in cancer [4]. It has a dual role in cancer, acting as both a tumor suppressor by preventing the accumulation of damaged proteins and organelles and as a mechanism of cell survival that can promote the growth of established tumors. Tumor cells activate autophagy in response to cellular stress and/or increased metabolic demands related to rapid cell proliferation. The role of autophagy in cancer has been highly researched and reviewed. There is evidence that emphasizes the role of autophagy both as a tumor suppressor as well as a factor in tumor cell survival. However, recent research has been able to show that autophagy is more likely to be used as a tumor suppressor [5].

Concerning hematological malignancies there is evidence suggests that autophagy defects in hematopoietic stem cells (HSCs) may be implicated in the pathogenesis of Myelodysplastic syndromes (MDS) and myeloproliferative disorders. Bone marrow cells from those patients are characterized by mitochondrial abnormalities and increased cell programmed death [3]. Indeed, multiple autophagic markers, including Beclin 1 and autophagy protein 7 have now been characterized as tumor suppressors [6], through controlling oxidative stress as well as the build-up of potentially DNA damaging wastes [7].

The evolution of autophagy and its relation to hematological malignancies; make it an encouraging field for research. Hoping for further progress in curative management of blood malignancies, so the modulation of autophagy is a promising potential strategy to enhance cancer therapy.

Ethical Approval: This study was approved by the Institutional Ethics Committee of School of Medicine, Minia University, Egypt, and all patients gave informed consent before participation in this study. The study conducted in accordance with the ethical guidelines of the 1975 Declaration of Helsinki and International Conference on Harmonization Guidelines for Good Clinical Practice.

Subjects and Method:

A total of 35 newly diagnosed patients with Myelodysplastic syndrome and myeloproliferative disorders collected in a single center, Minia University Hospital. The diagnosis of Myelodysplastic syndrome and MPNs was based on 2016 WHO criteria.

We excluded the Secondary causes of dysplasia from the MDS group.

During the same period, the serum of 35 apparently healthy individuals collected as a control group.

Clinical and laboratory assessments: The diagnosis of MDS is suspected clinically by symptoms and signs of anemia, bleeding tendency or repeated infections, and laboratory by the presence of an abnormal blood count at routine blood test. The assessment of dysplasia on PB and BM smears are the hallmark of the diagnosis of MDS according to the 2016 WHOcriteria.

Some additional laboratory tests were done to confirm the diagnosis in MPNs group as detection of JAK II mutation, serum erythropoietin level in polycythemia patients and BCR-ABL gene expression in CML patients.

Measurement of S. BECLIN-1and S. Atg-7: Using commercial kits from ANOVA Company:using Sandwich-ELISA technique. The Microelisa stripplate provided in this kit had been pre-coated with an antibody specific to Beclin 1and S. Atg-7. Then we calculated the concentration of Beclin 1 and Atg-7 in the samples by comparing the OD of the samples to the standered curve.

Assay Range: 30pg/ml- 2000 pg/ml.

Statistical Analysis: All analysis was carried out using SPSS software version 20. $P < 0.05$ was considered statistically significant.

Results

Out of 35 patients, 19 were males and 16 were females. Their age ranging from 30 to 70 years with 22 of them with no comorbidities but 13 of them had comorbidties. The control group contained 35 apparent normal individuals. Their age were ranging from 46 to 67, 20 of them were males and 15 were females. 31 of them with no comorbidities but 4 had comorbidities.

The patient group subdivided into two groups, 20 patients were MDS and 15 were MPNs. In the MDS: 10 were males and 10 were females. In MPNs: 9 were males and 6 were females. BM examination in MDS: 2 were hypocellular but 18 were hypercellular, while in MPNs: 5 were hypocellular and 10 were hypercellular. In MPNs, 1 case had mild splenomegaly, 4 had moderate splenomegaly and 10 had huge one.

Among the laboratory investigations between two groups: HB level in both groupsshowed statistically significant difference with P value <0.001*. Creatinine level, showed statistically significant difference between two groupswith P value <0.010*.By comparing serum beclin1 and Serum Atg 7in the two groups, there was statistically highly significant difference with P value <0.001

ROC curve for Beclin-1 showed area under curve 0.835, P value <0.001, sensitivity 94.3% and specificity 71.3%. while ROC curve for Atg 7 showed area under curve 0.962, P value <0.001, sensitivity 97.2% and specificity 94.3% . Using pearson correlation between S. Beclin1 and S. ATG7, there was a moderate association **(r=0.643 & p <0.001)**.

Table (1): Demographic data in patients and control group

Parameters	Case (n=35)	Control (n=35)	Test statistic	p value
Sex				
Male Female	19 (54.3%) 16 (45.7%)	20 (57.1%) 15 (42.9%)	χ^2 0.058	0.810
Age	58.5±8.9 (30-71)	57.8±6.8 (46-67)	0.379	0.706
Comorbidity				
No	22 (62.9%)	31 (88.6%)	6.293	0.012
Yes	13 (37.1)	4 (11.4%)		
Type of comorbidity				
HTN	6 (17.1%)	2 (5.7%)		
DM	4 (11.4%)	2 (5.7%)		
HTN/DM	1 (2.9%)	0		
Down/DM	1(2.9%)	0		
Asthmatic	1 (2.9%)	0		

Table (2): Laboratory investigations in both patients and control group

Parameters	Case (n=35)	Control (n=35)	t	p value
	Mean±SD (Range)	Mean±SD (Range)		
HB	7.6±4.1 (4-19)	11.5±0.9 (10-13)	-5.624	<0.001*
MCV	86.5±10 (59-101)	82.8±2.3 (80-86)	2.121	0.038*
PLT	214.7±353 (1-1321)	289.3±80.3 (178-411)	-1.220	0.227
TLC	9.5±13.4 (3-56)	7.7±1.9 (5-11)	0.777	0.440
INR	1.1±0.1 (1-1.3)	1.1±0.1 (1-1.3)	0.650	0.518
ALT	42.5±7.2 (35-60)	42.8±5.7 (35-55)	0.165	0.869
AST	45.9±8.4 (35-67)	47.6±5.9 (39-60)	-1.022	0.310
Cr	1±0.2 (1-1.4)	0.9±0.2 (1-1.2)	2.642	0.010*
beclin1	3223.9±1275.7 (14-7140)	1125.4±1423.3 (28-4215)	6.495	<0.001*
ATG7	3494.1±2841.8 (13-9870)	345.2±301.3 (15-1445)	6.519	0.001*

Figures:

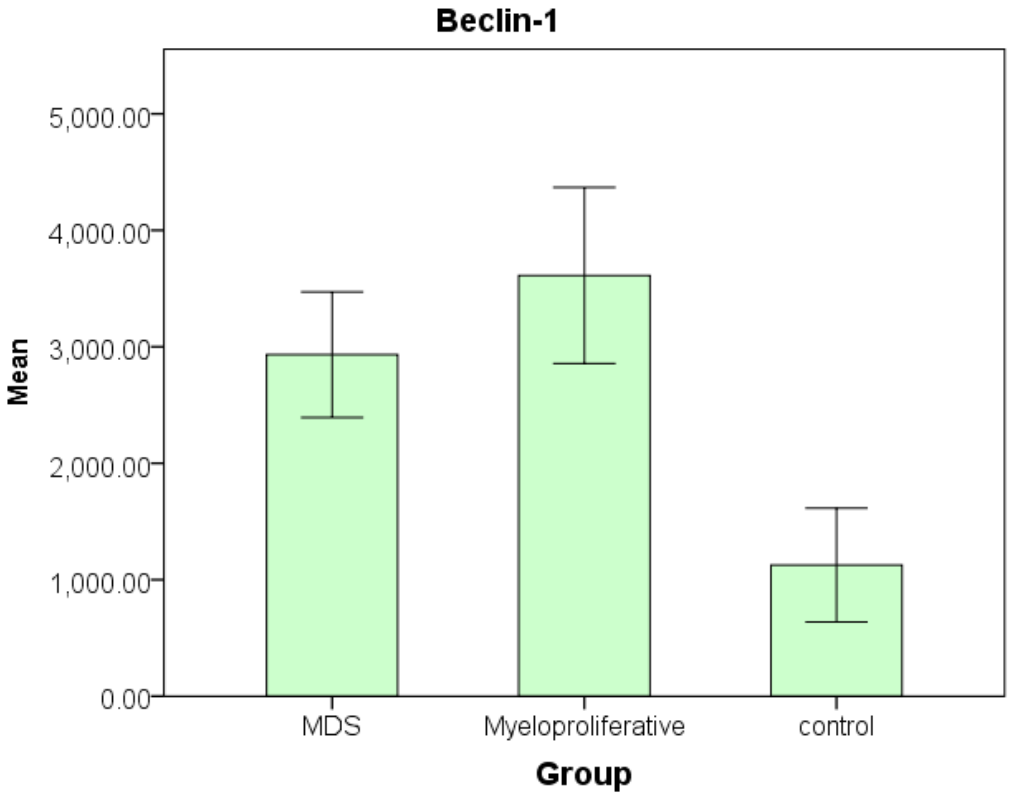

Figure (1): Comparison between S. Bcl-1 in different groups.

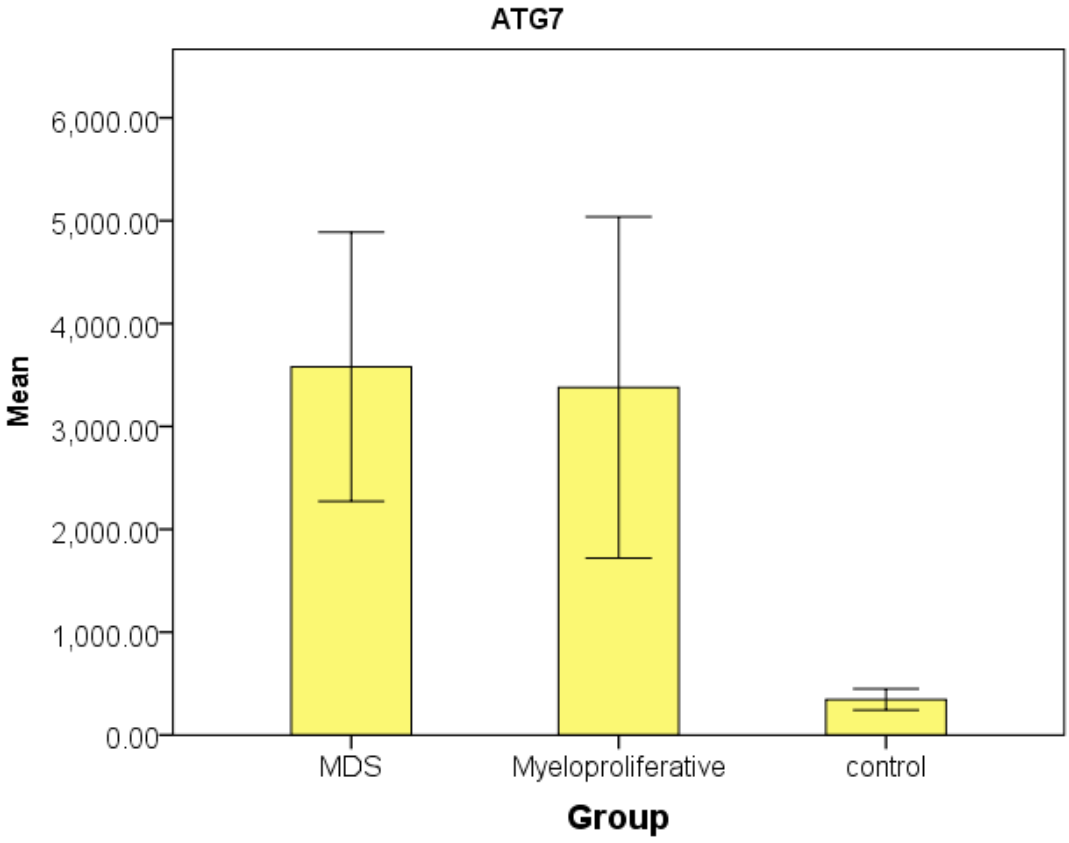

Figure (2): Comparison between S. ATG 7in different groups.

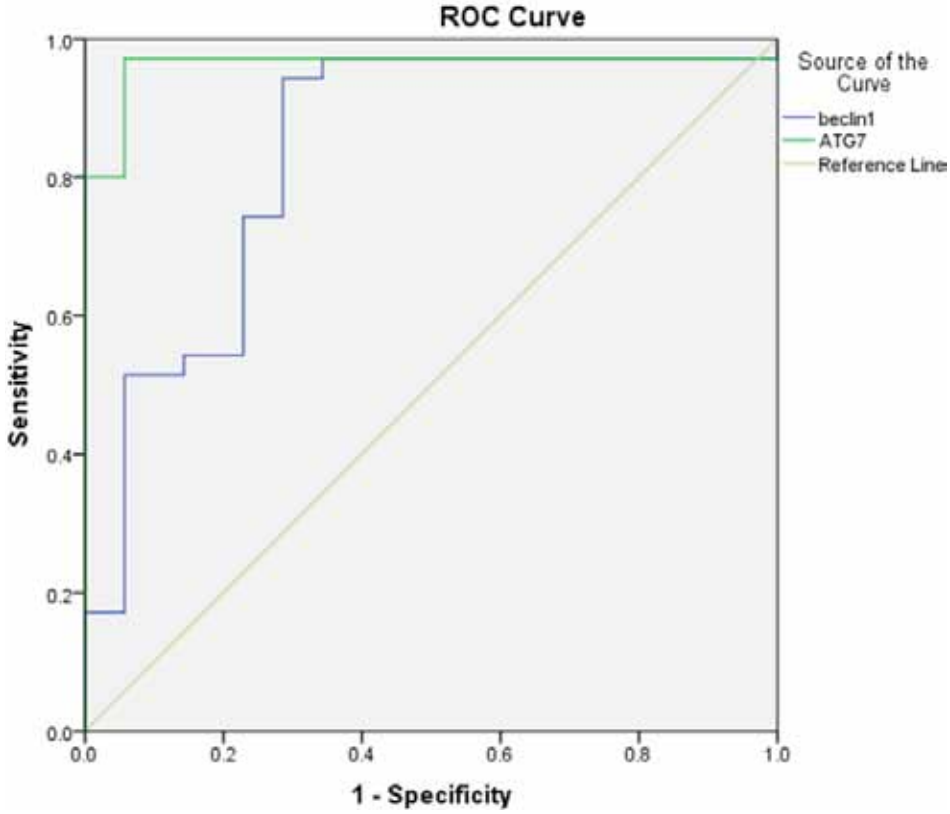

Figure (3): ROC curve forBcl-1 and ATG 7.

	Ares under the curve	P value	Cutoff point	Sensitivity	Specificity
Beclin1	0.835	<0.001	1160	94.3%	71.4%
Atg7	0.962	<0.001	435	97.2%	94.3%

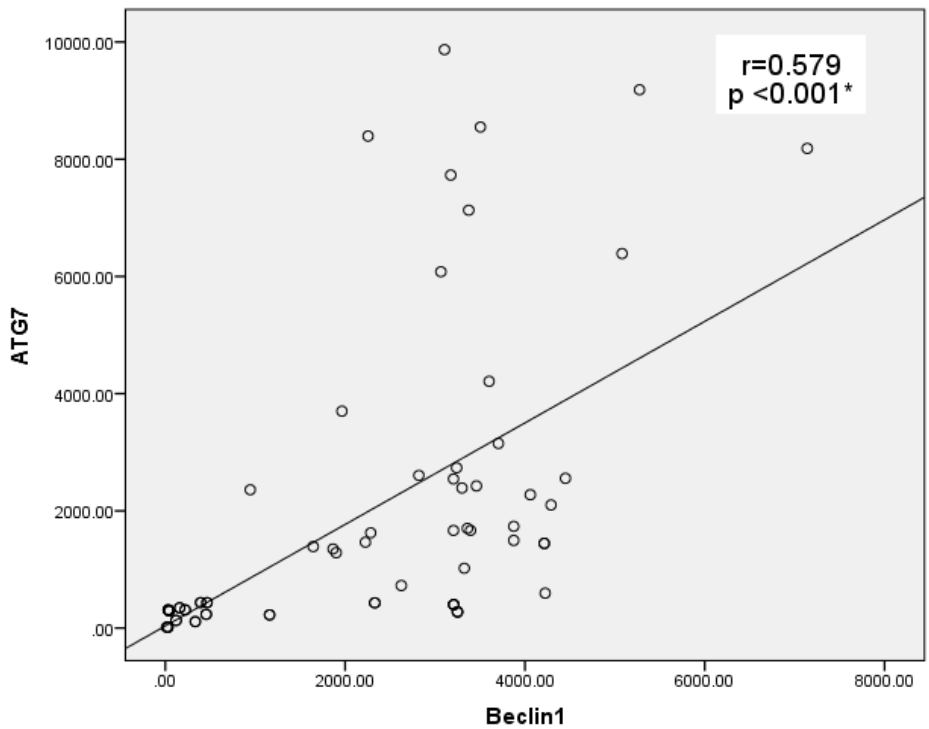

Figure (4): Correlation between Beclin 1 and ATG7 among the studied sample

Discussion

Autophagy is included in the regulation of haematopoietic stem cells (HSCs) that are the precursors of normal hematopoiesis[8,9]. Dysregulation of autophagy in HSCs is linked to the initiation and progression of blood cancers including: leukemias [10], lymphoproliferative disorders[11] and myelodysplastic syndrome[12]. Recently, many clinical trials using autophagy inhibitors are being applied to multiple blood cancer types together with chemotherapeutic agents to achieve complete cure. So it is the flavour of the moment to try to understand the relationship between autophagic protein markers and various blood malignancies.

In the present study, 20 newly diagnosed MDS patients and 15 patients of newly diagnosed MPNs, were assessed for autophagy by measuring serum beclin-1 and serum autophagy protien 7. The two groups of patients showed significantly increase in the level of S. BCL-1 and S. ATG-7 which indicating the induction of autophagy in the development of MDS and MPD.

Numerous recent studies suggest that inhibiting autophagy may be an efficient approach to improve the chemotherapeutic antileukemic regimens, but this was not in agreement with Alessio et al., [13], *whom* suggested that autophagy induction through using autophagy-activating pharmacological or dietary approaches could be utilized as a way to enhance the efficacy of chemotherapeutic agents as well as helping to reduce some of the side effects of anticancer agents.

Our data was agreed with Tan et al.,[14] as they proved that hypoxia-induced cell death increased upon knockdown of Beclin-1 or Atg7 as well as with autophagy-deficient cancer cell, were proliferating less in mouse xenograft models.

Folkerts et al.,[15] hypothesizedthat most of blood cancers induce autophagy which contributes to survival in poorly oxygenated tumor areas but, appropriate testing of the efficacy of autophagy inhibition in cancer cells of patients will have to be more developed, with current trials mainly monitoring autophagy in peripheral blood mononuclear cells (PBMCs) as a surrogate marker of response. However, the level of autophagy in PBMCs does not seem to correlate with autophagy inhibition in the tumor microenvironment [16]. Therefore, positron emission tomography/computed tomography and magnetic resonance imaging probes for Atg activity are currently being developed.

In our work, the serum BECLIN- 1, the key regulator of autophagy showed significantly increase in myeloproliferative group, especially in the CML subtype. These previous data was similar toXiaoli et al.,[17], whomdemonstrated that induction of upregulation of Beclin-1 help to augment autophagic cell survival. The levels of miR-93 depression and Beclin-1 upregulation contributed to the levels of Pediatric Leukemias (PL) resistance against chemotherapy. They suggested that strategies which increase miR-93 levels or inhibit cell autophagy may improve the outcome of PL, and this is our aim in the next studies to try the autophagy inhibitors and identify their role in cancer regression in combination with the currently used chemotherapeutic agents. Hoping to come in depth and more detailed informations about the role of autophagy in various blood malignancies, as It is evident that autophagy is more and more emerging as a potential target for cancer therapy.

Also,Vilcassim et al.,[18] were agreed with our results of high level of autophagic markers obtained in CML subgroup as they demonstrating thatiron chelator Deferasirox (DFX), blocked growth of myeloid leukemia cell lines whilst sparing normal stem cells through its ability to inhibit autophagy, postulating that modulation of intracellular iron levels can be adopted as a tool to elucidate the role of autophagy in this disease.

On the contrary, El-Sharkawy et al.,[19] elucidated that beclin-1 dependent autophagy is increased after the beginning of imatinib therapy indicating induction of autophagy in CML patients, along with complete clinical and hematological response, i.e. they supporting the theory of tumor suppression related autophagy and programmed cell death.

However, it is a must to take in our considerations that our study was carried out using serum autophagy markers by ELISA kits instead of gene expression, in a trial to facilitate and simplify the diagnosis and correlation between autophagy and blood malignancies.

However,Chen et al.,[20] hypothesized that irregular myeloid proliferation occurred in the bone marrow of autophagy related gene (Atg) 7 knockout mice, which was similar to the process of development of myelodysplastic syndrome (MDS) and this support the theory that the presence of autophag guard against tumor development.

In the current study, there is significant increase

in both S. Atg 7 and S. BECIN-1 in MPNs group compared to the control group that was in accordance withCellsoão et al.,[21] who reported that it was better to combine Ruxolitinib; which is a JAK2 inhibitor used in MPNs with positive JAK2 mutation, with autophagy pharmacological inhibitors, especially chloroquine, may be a promising strategy for improving the outcome of MPN with positive JAK2 mutation. This supports the theory that autophagy promotes blood malignancies.

Limitations of the present study: (1) The number of subjects that was small due to financial issues. (2) Future studies are required to further using pharmacological autophagy inhibitors in a larger sample size and assess its role in regression of the blood malignancies either alone or in combination with available chemotherapeutic agents. (3) S. BECN-1 and S. Atg 7 levels were not assessed after initiation of treatment which could provide important information about the relation between autophagy and response to chemotherapy.

Conclusion

High level of serum BECN-1 and serum Atg 7 in MDS and MPNs indicate that they can be autophagy-dependent markers and it may be a therapeutic window for autophagy inhibition in combination with cancer therapy.

Acknowledgement:

Ethical Statement: The material has not been published anywhere. Authors of the manuscript have no financial ties to disclose and have met the ethical adherence.

Disclosure of Interest: The authors declare that they have no competing interests.

Declaration of Authorship: All authors have directly participated in the planning, execution, analysis or reporting of this research paper. All authors have read and approved the final version of the manuscript.

Conflict of Interest: None

References

1. "The Nobel Prize in Physiology or Medicine 2016". The Nobel Foundation. 3 October 2016. Retrieved 3 October 2016.
2. Watson AS, Mortensen M and Simon AK. Autophagy in the pathogenesis of myelodysplastic syndrome and acute myeloid leukemia. Cell Cycle.2011;10:1719-1725.
3. Zhineng J. Yang, Cheng E. Chee, Shengbing Huang, et al. The Role of Autophagy in Cancer: Therapeutic ImplicationsMol Cancer Ther 2011;10:1533-1541.
4. Rosenfeldt MT, Ryan KM. The multiple roles of autophagy in cancer. Carcinogenesis 2011; 32 (7): 955–963.
5. Antonioli M, Di Rienzo M, Piacentini M, et al. Emerging Mechanisms in Initiating and Terminating Autophagy. Trends Biochem Sci 2016; 968: 30171-30182.
6. Dalby KN, Tekedereli I, Lopez-Berestein G, et al. targeting the prodeath and prosurvival functions of autophagy as novel therapeutic strategies in cancer. Autophagy.2010; 6:322-329.
7. Hu YL, DeLay M, Jahangiri A, et al. Hypoxia-induced autophagy promotes tumor cell survival and adaptation to antiangiogenic treatment in glioblastoma. Cancer Res. 2012;72:1773–83.
8. S. Bomken, K. Fiˇser, O. Heidenreich, et al. "Understanding the cancer stem cell," British Journal ofCancer, 2010; 103, 4: 439–445,
9. J. E. Crowley, J. L. Scholz,W. J. Quinn III et al., "Homeostatic control of B lymphocyte subsets," Immunologic Research, 2008; 42: 75–83,
10. Garg AD, Dudek AM, Ferreira GB, et al. ROS-induced autophagy in cancer cells assists in evasion from determinants of immunogenic cell death. Autophagy.2013;9:1292-1307.
11. Watson AS, Mortensen M and Simon AK. Autophagy in the pathogenesis of myelodysplastic syndrome and acute myeloid leukemia. Cell Cycle.2011;10:1719-1725.
12. Evangelisti C, Evangelisti C, Chiarini F, et al. Autophagy in acute leukemias: A double-edged sword with important therapeutic implications. Biochim Biophys Acta.2015;1853:14-26.
13. Alessio Nencioni, Michele Cea, Fabrizio Montecucco, et al. Autophagy in blood cancers: biological role and therapeutic implications haematologica 2013; 98(9)
14. Tan Q, Wang M, Yu M, et al. Role of autophagy as a survival mechanism for hypoxic cells in tumors. Neoplasia. 2016; 18:347- 355.
15. Folkerts, H., Hilgendorf, S., Vellenga, E., et al.

The multifaceted role of autophagy in cancer and the microenvironment. Medicinal research reviews. (2018) DOI:10.1002/med.21531

16. Mahalingam D, Mita M, Sarantopoulos J, et al. Combined autophagy and HDAC inhibition: a phase I safety, tolerability, pharmacokinetic, and pharmacodynamic analysis of hydroxychloroquine in combination with the HDAC inhibitor vorinostat in patients with advanced solid tumors. Autophagy. 2014; 10(8):1403-1414.
17. Xiaoli Wua, Xuefeng Fengb, Xiaoqing Zhaoa,et al. Role of Beclin-1-Mediated Autophagy in the Survival of Pediatric Leukemia Cells Cell Physiol Biochem 2016; 39:1827-1836
18. F. S. Vilcassim, A. Banerjee, G. Grigoriadis. THE IMPACT OF AUTOPHAGY ON THE PROGRESSION OF THE MYELODYSPLASTIC SYNDROMES. Poster Presentations – 14th International Symposium on Myelodysplastic Syndromes/Leukemia Research 55 S1. 2017; S45–S167
19. El-Sharkawy EA, Salah KM, Kamal Eldin AM, et al. Clinical Significance of Beclin-1 Dependent Autophagy Induced by Imatinib in Chronic Myeloid Leukemia Patients. J Mucosal Immunol Res 2017;1: 1000103.
20. Y.-F. Chen, Liu, Luo, et al. The roles of reactive oxygen species (ROS) and autophagy in the survival and death of leukemia cells. Critical Reviews in Oncology/Hematology .2017; 112: 21–30
21. Cellsoão Agostinho Machado-Neto, BSc, Juan Luiz Coelho-Silva, et al. Autophagy Inhibition Potentiates Ruxolitinib-Induced Apoptosis in $JAK2^{V617F}$ cells, ASH annual meeting. December, 2018.