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ORIGINAL ARTICLE

Galectins in acute myeloid leukemia: gene expression profiling with clinical emphasis

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Summary

Purpose: *Galectins family are animal lectins with a broad range of cellular functions. Many galectins are repeatedly reported in several physiological changes and diseases including cancers. In acute myeloid leukemia (AML), there is a big focus on galectins 3 and 9, but not the other galectins.*

Methods: *Bone marrow (BM) and corresponding peripheral blood (PB) were collected from recently diagnosed 45 adult patients with de novo AML and 8 normal individuals. The profiling was done using qRT-PCR for eight members of the galectins' family.*

Results: *Our results showed dysregulation of several galectins in AML patients. Upregulation of galectin 1 has shown a significant correlation to monocytic AML, as it was more upregulated in M4 and M5 (p=0.015 and p=0.006 in peripheral blood and bone marrow respectively), as well as positive CD4, CD11c, and CD64. The same finding was encountered with galectin 2 where its overexpression was also a sign of monocytic AML. The other galectins are statistically significant with many clinicopathological features indicating their clinical significance. The expression of MHC class II is significantly associated with overall survival (OS) advantage (p<0.001).*

Conclusions: *Galectin 1 and 2 could be markers for monocytic AML. The presence of MHC class II may be good prognostic factor and showing higher overall survival.*

Key words: *galectins, AML, bone marrow, peripheral blood, qRT-PCR*

Introduction

Acute myeloid leukemia (AML) is a heterogeneous disease of haemopoietic stem cells, characterized by inhibition in the differentiation of hematopoiesis, causing accumulation of blasts at various stages of differentiation. Consequently, it ends up with reduced production of healthy haemopoietic elements [1]. AML blasts have different sizes which can be slightly larger than lymphocytes. Nuclei of blast cells are larger, have various shapes and usually comprise several nucleoli [2]. In the last three decades, combination of anthracycline-like daunorubicin or idarubicin, and cytarabine were utilized as AML treatment [3].

Galectins are a family of animal lectins (carbohydrate-binding proteins) comprising 15 members. Each galectin has one or two carbohydrate-recognition domains (CRDs) with an affinity for β-galactosides. Galectins are classified according to their structure and number of CRDs into three different categories:(a) prototype galectins, comprising 1, 2, 5, 7, 10, 11, 13, 14, and 15 which have one CRD, (b) tandem-repeat galectins 4, 6, 8, 9 and

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12 which contain two homologous CRDs connected by linker domains, within a single polypeptide and (c) chimeric type (galectin 3) which consists of one CRD linked to a non-lectin N-terminal domain [4]. Galectins are involved in several cellular functions including cell signaling, proliferation, migration, apoptosis and cancer progression [5].

Our study was carried out to investigate the dysregulation of galectins expression in bone marrow and corresponding peripheral blood samples of AML diagnosed patients and correlating them to clinicopathologic data.

Methods

Study hypothesis

This study was based on two previous projects. The original hypothesis was to investigate galectin 4 and its expression in peripheral blood to track any interaction with red blood cells as investigated *in vitro* [6]. Also, the present work was scheduled to get more information about galectins in bone marrow (BM) of AML patients after our previous findings in peripheral blood [7].

Before starting this project, ten random peripheral blood samples of different hematologic cancers were collected. Then, the immunostaining of galectin-4 was performed as described previously [6] (Supplementary Figure S1). Interestingly, a different range of galectin 4 upregulation was recorded in six out of ten patients conferring a potential role of galectin(s) in the peripheral blood of hematologic malignancies.

As it is very expensive to track the protein level we investigated the mRNA of galectins-related in AML patients utilizing qRT-PCR technology and correlate the expression with clinical data.

Patients and samples

BM and corresponding peripheral blood (PB) were collected from 45 adult patients (16 females and 29 males) with the average age of 41.5 years diagnosed with *de novo* AML in the National Cancer Institute (NCI), Cairo University (CU). All patients were classified morphologically and immunologically (Table 1). From each patient or their legal guardians, written informed consent was signed. Our study was approved by the ethical committee of the National Cancer Institute Cairo University (201617027-4). BM and PB samples were also collected from 8 healthy volunteers. The eight normal samples were from the same hospital and included patients with negative cancer results or volunteers for BM transplantation.

RNA purification and qRT-PCR

Total RNA purification from BM and PB was performed using TRIZOL reagent (Life Technologies). cDNA synthesized was performed using the High-Capacity cDNA Reverse Transcription Kit. PCR was performed for Galectins 1, 2, 3, 4, 8, 9, 12, and 13 and GAPDH genes using specific primers (Supplementary Table S1). The CT (cycle threshold) values were collected and normalized to a house keeping gene (GAPDH). $2^{-\Delta\Delta CT}$ was used to calculate the relative fold changes in comparison to normal individuals.

Statistics

Statistical analyses were carried out using IBM SPSS version 24. Mann-Whitney/Kruskal Wallis statistical tests were performed for the expression data across the different clinical subgroups of patients. Overall survival in patients' group were calculated using the Kaplan-Meier method.

Table 1. Clinicopathologic parameters of the investigated AML cohort

Parameter	*n (%)*	
Gender		
Male	29 (64.4)	
Female	16 (35.6)	
Diagnosis		
M1	7 (15.9)	
M2	20 (45.5)	
M4	11 (25)	
M5	6 (13.6)	
Age (years)		
≤42 (median value)	23 (51.1)	
>42 (median value)	22 (48.9)	
FLT3 (ITD)		
Wild	34 (85)	
Mutant	6 (15)	
FLT3 (TKD 835)		
Wild	37 (94.4)	
Mutant	2 (5.1)	
Cytogenetics		
t(8;21) -ve, Inv(16) -ve	18 (78.3)	
t(8;21)+ve, Inv(16) -ve	2 (8.7)	
t(8;21) -ve, Inv(16) +ve	3 (13.0)	
IPT	Present	Absent
CD34	25 (62.5)	15 (37.5)
CD117	29 (72.5)	11 (27.5)
CD19	3 (7.5)	37 (92.5)
CD22	1 (2.5)	39 (97.5)
CD16	1 (2.5)	39 (97.5)
CD7	1 (2.5)	39 (97.5)
CD56	5 (12.5)	35 (87.5)
CD64	14 (35)	26 (65)
CD4	7 (17.5)	33 (82.5)
CD8	1 (2.5)	39 (97.5)
CD11c	9 (22.5)	31 (77.5)
CD14	7 (17.5)	33 (82.5)
CD20	1 (2.5)	39 (97.5)
CD61	1 (2.5)	39 (97.5)
MHC CLASS II	29 (64.4)	11 (24.4)
Splenomegaly	8 (17.8)	37 (82.2)
Hepatomegaly	13 (28.9)	32 (71.1)

Results

Galectins expression in AML

The profiling of eight members of the galectin's family in PB cells and BM of AML patients compared to normal individuals was performed. A cut off value of 4 for fold change was considered, and data between 4 and -4 were considered as normal expression. Above and below 4-fold change, the gene expression was considered dysregulated.

In PB, *LAGALS3* expression exhibited the most downregulation in 73.3% of patients, followed by *LAGALS2* and *LAGALS12* in 42.2%, then *LAGALS13* with 28.8%, then *LAGALS8* with 15.5%, then *LAGALS9* with 13.3%, then *LAGALS1* 8.8% and then *LAGALS4* with 6.66%. Accordingly, galectin 4 exhibited the most up-regulation in PB with 60%.

In BM cells *LAGALS3* also exhibited the most downregulation in 62.2%, followed by *LAGALS2* in 31.1%, then *LAGALS13* in 13.3%, then *LAGALS9* in 6.6%, then *LAGALS12* in 4.4%, then *LAGALS1* and *LAGALS4* in 2.2%, and *LAGALS8* with no downregulation (Figure 1).

Galectin expression and clinicopathologic data

The expression of each of the eight selected galectins was compared to all of clinical characteristics for each patient's BM and PB samples. Mann Whitney/Kruskal Wallis statistical tests were performed for the expression data across the different clinical subgroups of patients (Table 2).

In PB we found a statistically significant association between the expression of *LGALS1* and FAB (French–American–British classification of acute myeloid leukemia), and higher expression was associated with M4 and M5 (p=0.015). Also, galectin 1 was found to be significantly related with different surface antigens as follows: higher expression was accompanied with positive CD64 (p=0.015), the presence of CD4 (p=0.011), CD14 (p=0.018) and CD11c (p=0.003). Furthermore, higher regulation was associated with positive MHC class II (p=0.030) (Figure 2).

In BM cells, *LGALS1* was statistically significant with FAB classification of AML, and higher expression was associated with M4 and M5 (p=0.006). Also, the upregulation was associated with the presence of CD4 (p=0.038) and CD11c (p=0.011) (Figure 2).

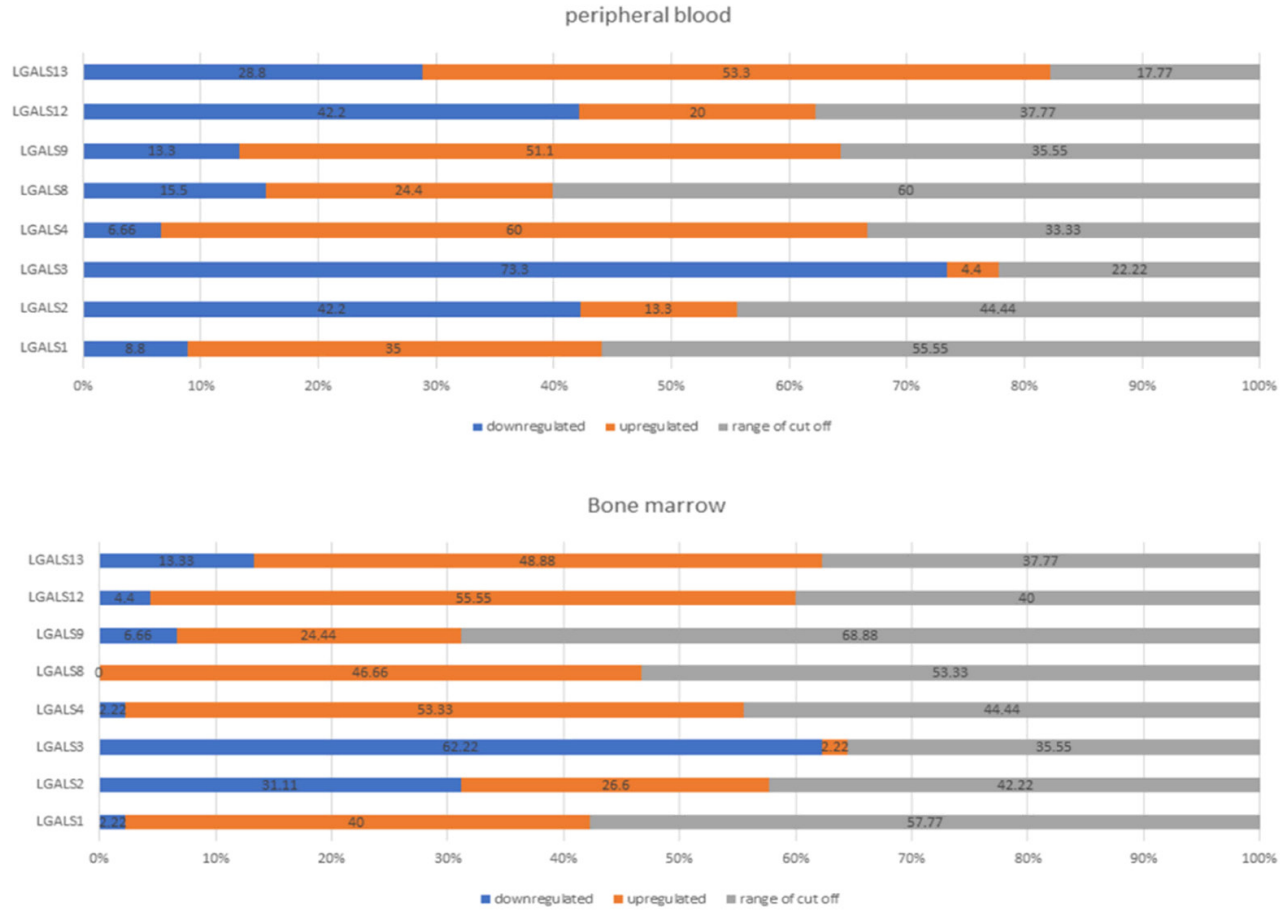

Figure 1. Galectin's expression pattern in AML patients. Histograms are showing the percentage of patients exhibiting dysregulation in galectins in peripheral blood and bone marrow.

Table 2. Galectin's expression analysis with different clinical parameters using Mann Whitney/Kruskal Wallis statistical tests

Clinical data	*GAL 1*		*GAL2*		*GAL3*		*GAL 4*		*GA8*		*GAL9*		*GAL12*		*GAL13*	
	BM	*PB*	*BM*	*PB*	*BM*	*PB*	*BM*	*PB*	*BM*	*PB*	*BM*	*PB*	*BM*	*PB*	*BM*	*PB*
Gender	0.2	0.943	0.079	0.291	0.107	0.177	0.602	0.776	0.477	0.420	0.53	0.602	0.514	0.387	0.934	0.868
Age	0.577	0.542	0.114	0.128	0.28	0.858	0.856	0.290	0.716	0.982	0.724	0.699	0.562	0.617	0.856	0.471
Diagnosis (M1-M2-M4-M5)	0.024	0.103	0.061	0.055	0.122	0.075	0.474	0.209	0.404	0.571	0.847	0.671	0.051	0.750	0.036	0.212
Diagnosis (M1+M2)/(M4+M5)	0.006	0.015	0.007	0.008	0.027	0.012	0.492	0.159	0.341	0.462	0.386	0.368	0.656	0.294	0.621	0.159
Initial BM%	0.301	0.733	0.8	0.233	0.401	0.440	1	0.594	0.666	0.601	0.767	0.351	0.524	0.082	0.682	0.900
FLT3 (ITD)	0.161	0.520	0.52	0.747	0.191	0.820	0.596	0.880	0.344	0.449	0.075	0.636	0.344	0.185	0.94	0.791
FLT3 (TKD 835)	0.524	0.445	0.34	0.610	0.924	1.000	0.849	0.874	0.702	0.340	0.339	0.949	0.524	0.111	0.799	0.750
Cytogenetics (Inv16)	0.423	0.365	0.01	0.174	0.098	0.500	0.698	0.940	0.482	0.922	0.048	0.530	0.856	0.419	0.655	0.610
CD34	0.52	1.000	0.922	0.769	0.685	0.567	0.989	0.321	0.15	0.328	0.706	0.706	0.349	0.823	0.349	0.317
CD117	0.126	0.062	0.94	0.178	0.348	0.188	0.844	0.047	0.639	0.060	0.844	0.209	0.458	0.220	0.55	0.317
CD19	0.174	0.317	0.7	0.292	0.898	0.342	0.208	0.681	0.248	0.980	0.898	0.939	0.59	0.369	0.662	0.369
CD22	0.129	0.516	0.319	0.209	0.516	0.516	0.209	0.573	0.179	0.209	0.109	0.516	0.129	0.573	0.13	0.153
CD16	0.129	0.242	0.461	0.634	0.386	0.897	0.829	0.279	0.319	0.829	0.462	0.319	0.829	0.897	0.319	0.897
CD7	0.662	0.397	0.208	0.626	1	0.085	0.7	0.130	0.857	0.898	0.739	0.739	0.626	0.898	0.426	0.397
CD56	0.919	0.683	0.698	0.728	0.668	0.500	0.951	0.581	0.951	0.984	0.553	0.581	0.487	0.379	0.474	0.838
CD64	0.207	0.015	0.092	0.047	0.239	0.084	0.755	0.788	0.533	0.660	0.821	0.228	0.223	0.788	0.148	0.321
CD4	0.037	0.003	0.569	0.122	0.206	0.084	0.817	0.328	0.072	0.126	0.957	0.144	0.233	0.206	0.278	0.346
CD8	0.091	0.109	0.386	0.762	0.153	0.319	0.363	0.319	0.634	0.762	0.634	0.697	0.573	0.697	0.897	0.634
CD11c	0.011	0.003	0.003	0.006	0.016	0.089	0.549	0.030	0.264	0.384	0.356	0.582	0.278	0.250	0.808	0.884
CD14	0.072	0.018	0.144	0.072	0.14	0.020	0.845	0.618	0.364	0.749	0.581	0.455	0.606	0.278	0.488	0.016
CD20	0.319	0.363	0.762	0.363	0.697	0.829	0.242	0.965	0.209	0.829	0.209	0.410	0.516	0.109	0.091	0.461
CD61	0.319	0.363	0.762	0.363	0.697	0.829	0.242	0.965	0.209	0.829	0.209	0.829	0.516	0.109	0.091	0.461
MHC CLASS II	0.13	0.030	0.289	0.041	0.130	0.099	0.774	0.030	0.683	0.220	0.555	0.042	0.617	0.025	0.916	0.639
Blood Group	0.953	0.310	0.8	0.481	0.427	0.467	0.238	0.591	0.867	0.937	0.449	0.302	0.432	0.853	0.306	0.803
Splenomegaly	0.778	0.306	0.031	0.191	0.025	0.116	0.882	0.279	0.678	0.449	0.389	0.449	0.306	0.964	0.593	0.272
Hepatomegaly	0.9	0.244	0.625	0.920	0.416	0.193	0.582	0.634	0.023	0.388	0.689	0.416	0.193	0.475	0.423	0.057

In peripheral blood Galectin 2 was statistically significant with FAB classification, and higher expression was associated with M4 and M5 (p=0.008). The higher expression of galectin 2 was also associated with positive CD64 (p=0.047), CD11c (p=0.006), and MHC class II (p =0.041). In BM cells, galectin 2 was shown to be statistically significant with cytogenetics, and higher expression was associated with the presence of Inv16 (p=0.016). In addition, the mRNA elevation was related to CD11c (p=0.003) and splenomegaly (p=0.031) (Figure 3).

LGALS3 higher expression was associated with the presence of CD14 (p=0.020) in PB, whereas, in BM, a high level of *LGALS3* was associated with positive CD11c (p=0.016) and splenomegaly (p=0.025).

Positive expression of galectin 4 in peripheral blood was statistically significant with CD117, in which the higher expression was associated with the positive CD117 (p=0.047), CD11c (p=0.030), and MHC class II (p=0.030). Downregulation of galectin 8 in BM was shown to be associated with hepatomegaly (Supplementary Figure S2). Peripheral blood expression of galectin 8 was not affected by the presence of hepatomegaly, also no survival advantage was obtained even when we included the result of the previous cohort.

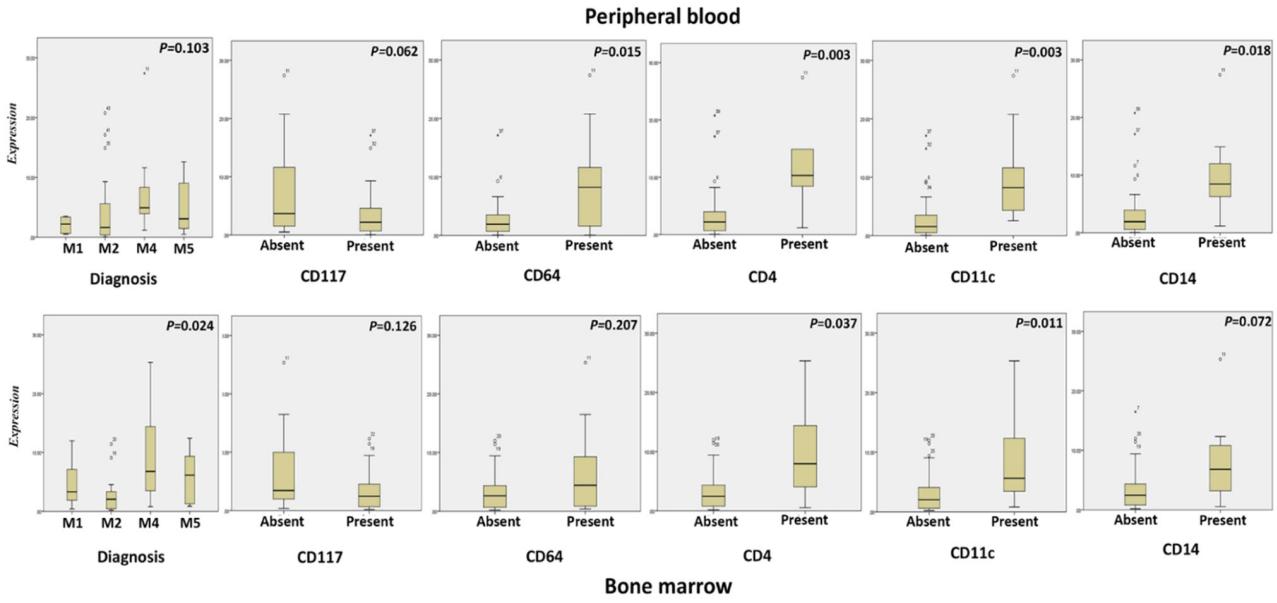

Figure 2. Galectin 1 expression and clinicopathological data in peripheral and bone marrow in AML patients.

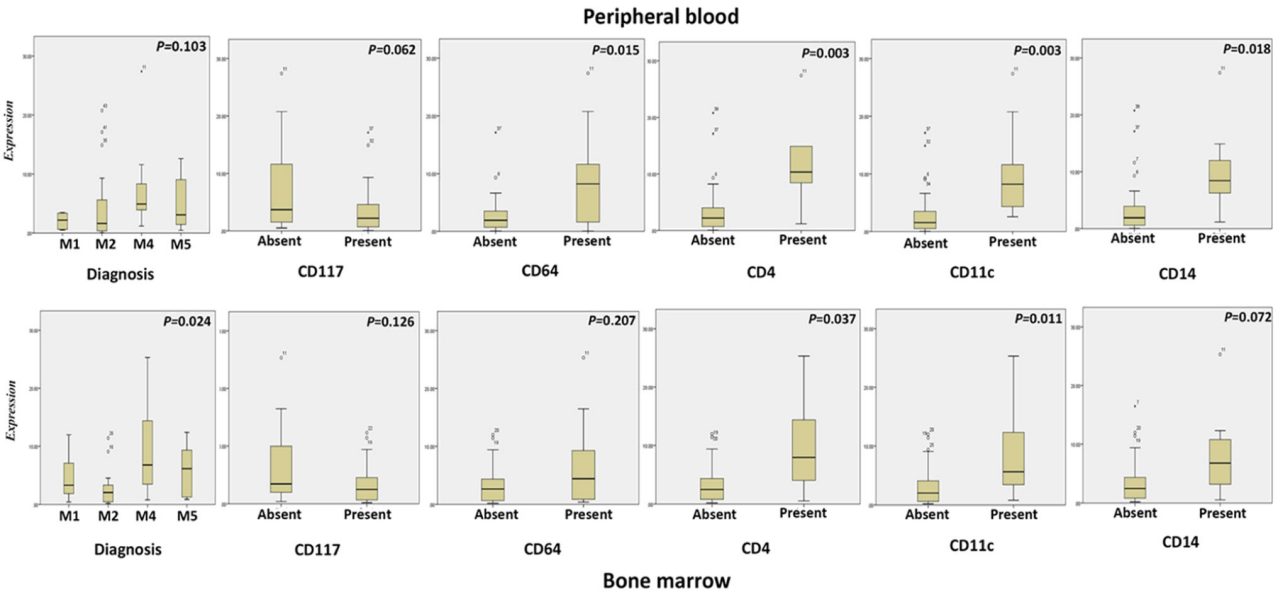

Figure 3. Galectin 2 transcription is represented as box plots and different clinical data.

Our data show a statically significance association between higher expression of galectin 9 in PB and the presence of MHC class II (p=0.042). In the BM, *LGALS9* was statistically significant with cytogenetic data, in which higher expression was associated with Inv (16) +ve (p=0.048) as shown in Supplementary Figure S3.

LGALS12 in PB was statistically significant with MHC class II, where the upregulation of galectin 12 was associated with MHC class II (p=0.025).

In PB, *LGALS13* was significant with CD14, where lower expression was correlated to positive CD14 (p=0.016). In the BM, *LGALS13* was significant with FAB classification (p=0.036).

Spearman Rho correlation analysis

Correlation analysis was performed for the expression of each galectin in BM and corresponding PB. The expression of each galectin in the BM and peripheral blood was significant except galectins 4, 9, 12. The results are shown in Supplementary Table S2. Also, the ratio of expression fold change in PB/BM was also calculated and compared in different patients' samples as shown in Supplementary Figure S4.

Discussion

AML is more frequently seen in older adults [8]. Around 52% of adult primary AML patients bear non-random chromosomal abnormalities which cause and promote the disease [9]. AML patients with particular cytogenetic abnormalities including inv(16), t(9;11) and t(15;17) are associated with longer remission and survival [10]. As stated by National Cancer Registry Program from 2008 to 2011, the incidence of myeloid leukemia in Egypt in patients aged 18 years or more was 26.7/100 000 in men and 33.5/100 000 in women. In females, they constitute 6.5% which is second after breast cancer, while in males are fourth after liver cancer [11].

The present study is a follow-up of a previous work published in 2015 [7] using PB samples only of AML and the result was found in *LGALS4* and *LGALS12*. The higher expression of *LGALS4* has been reported to have significant association with younger AML patients. A statistically significant difference between OS and *LGALS12* gene has been found, and elevation of *LGALS12* was related to higher overall survival.

Hereby, for galectins expression profiling, a different cohort of clinical samples was analyzed as a follow-up study. In our published paper in 2015 [7] the PB samples of AML patients were used. One of these encountered drawbacks in our previous study was the absence of enough sampling data for each patient since we missed the BM samples and some of the surface antigens data. Hence, here in this study, a new cohort was used, in which PB and BM samples were analyzed for each patient and a healthy individual. In our present study, the expression of most galectins in PB was correlated to the level in the BM, except galectins 4, 9, and 12.

Dysregulation of galectins expression in tumor microenvironment was reported in recent research and indicate important role in cancer. The increased galectin 1 expression has been correlated with many types of cancerous tissues including pancreas, breast, prostate, lymphoma and leukemia. Elevation of galectin 1 is involved in cancer development and progression [12,13]. RAS proteins are proto-oncogene products that transmit signals for cell division to control cellular proliferation, differentiation, cell migration and apoptosis [14]. Galectin 1 interacts with H-Ras-GTP through an interaction between the hydrophobic pocket in galectin 1 and farnesyl group of RAS, enhances the activity of RAS signal which activates increase of cell transformation [15,16]. Galectin 1 is recruited to plasma membrane from cytosol and interacts with H-Ras-GTP increasing the stability of H-Ras-GTP and its membrane association [15].

A single amino acid mutation (L11A) in hydrophobic pocket of galecin 1 results in deactivation of active H-Ras activity [16]. Galectin 1 mediated immunosuppression by T cell apoptosis through its effects on CD4+ and CD8+ [17]. Besides, with its immunosuppressive function, galectin 1 converts vascular endothelial growth factors (VEGFs) and Neuropilin 1 (NRP-1) and promotes the growth of new capillaries (angiogenesis)[18].

Our results have a clear pattern about galectin 1 expression in monocytic AML. The high expression of galectin 1 was found in M4 and M5 (FAB classification). Moreover, the presence of CD4, CD14, CD64 and CD11c is significantly associated with high expression of galectin 1. According to our knowledge, the association of upregulation of galectin 1 and monocytic leukemia is the first time to be reported here. Even though, a larger cohort has to be studied to confirm this association.

Galectin 2 has a single-CRD and primarily expressed in GIT. From the functional point of view, galectin 2 has a pro-apoptotic role on activated T cells depending on intrinsic apoptotic pathways via caspase-3 and caspase-9. The process of apoptosis activation occurs in the absence of binding to the glycoprotein CD3 or CD7 [19]. Galectin 2 expression was shown to be decreased in gastric cancer progression, reflecting a potential tumor suppressive function [21]. In our data here, galectin 2 over-

expression had an association with the presence of some markers of monocytic AML.

Galectin 3 is the only chimera-type galectin, encoded by *LGALS3* (14q21-22), and is a 29-35KDa. It has a wide range of cellular activities involved in carcinogenesis, invasion and metastasis of several types of cancers including blood cancers [22,23]. Depending on the types of cancer and subcellular localization, galectin 3 could have contradictory influence on cancer development [24]. Overexpression of galectin 3 was repeatedly reported in different cancers to function in tumor progression and metastasis [25-27]. On the other hand, downregulation of galectin 3 was reported in breast [28-30], gastric [31] and prostate cancers [32]. In AML, galectin 3 upregulation was repeatedly reported and indicates a bad prognostic factor [33]. Our finding showed a clear down-expression which implies a good prognostic marker but this was not confirmed by our statistical analysis which could be due to ethnic population and that was the same result of our previous study [7] in ALL. Therapeutic inhibition of galectin 3 was proposed to improve outcome [34].

In 20 out of 45 patients, galectin 4 expression level was higher in peripheral blood than in BM in the same individual. According to a previous study, galectin 4 was increased upon incubation of pancreatic cell line with blood *in vitro* [6]. This elevated level of expression could reflect the role of galectin 4 in tumor cell relapse and interactions with the blood cells in cancer.

Galectin 8 functions in several cellular processes including cell growth, adhesion, spreading and apoptosis [35]. It has a pro-apoptotic effect on activated T cells [36]. The amount of galectin 8 decreased in tumor tissue compared to normal in the colon, pancreas, liver, skin, and larynx. Conversely, galectin 8 increased in breast and remained unchanged in the lung, bladder, kidney, prostate and stomach [37]. In our study galectin 8 didn't show any downregulation and it was upregulated in 64.6% of the patient's samples.

In AML, cells secrete high levels of Tim-3 and its ligand galectin 9 which suppress the activity of NK cells and cytotoxic T cells [38]. Galectin 9 and Tim-3 secretion is induced by mTOR pathway which inhibits the secretion of IL-2 from activated T cells [39]. In our study elevation of galectin 9 in peripheral blood was associated with the presence of MHC class II which strengthens the immunosuppressive function of galectin 9. Interestingly, galectin 9 is more expressed in PB than in BM which could also support its role in PB.

Galectin 12 is expressed in macrophages and effects inflammation and macrophage polarization and activation [40]. *In vitro*, galectin 12 expression has been reported to induce G1 arrest and apoptosis [41]. In our previous study [7] galectin 12 was mostly downregulated and this down expression was found to be associated with lower survival. We didn't get the same result in the present study. Hereby, we found more expression of galectin 12 in BM compared to PB, contrary to galectins 4 and 9.

In the present study the expression of MHC class II on AML blast provided a significant OS advantage ($p<0.001$) as shown in Supplementary Figure S5. To the best of our knowledge this finding was not reported before, perhaps because MHC class II is involved with better presentation of endogenous antigens to the host immune system. Moreover, here we reported a significant association between the presence of MHC class II on AML blasts and higher expression of galectins 1,2,4,9,12 ($p=0.030$, $p=0.041$, $p=0.030$, $p=0.042$, $p=0.025$) in PB expression only, but not when their BM counterparts were considered. Conversely, splenomegaly in our AML cases was significantly associated with higher BM expression of both galectin 2 and 3, but not when their PB expression was considered.

In the present cohort, neither splenomegaly nor hepatomegaly provided any significant survival advantage. Here we showed a significant association between hepatomegaly and both decreased expression of BM galectin 8 ($p=0.023$) and increased expression of PB galectin 13 ($p=0.057$) only.

In conclusion, galectins' family is dysregulated in AML. Galectin 1 and 2 could be markers for monocytic AML. Galectin 9 is more upregulated in PB than in the BM of the same patient, reflecting a role in blood. MHC class II is influenced by higher expression of five peripheral blood galectins (1, 2,4,9,12). Inversion 16 is associated with higher BM expression of galectin 2 and higher PB galectin 9. Other galectins are also associated with the presence of different clinical factors. This study and our previous studies emphasize the role of galectins in cancer. A deeper understanding is still needed by investigating a larger cohort.

Additional information

Supplementary Figures and Tables are available at: www.jbuon.com/archive/26-6-C26471-Supplementary-materials.pdf.

Conflict of interests

The authors declare no conflict of interest. Our study was approved ethical committee of national cancer Institute Cairo University.

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