ISSN NO. 2320-5407

Journal homepage: http://www.journalijar.com

INTERNATIONAL JOURNAL OF ADVANCED RESEARCH

RESEARCH ARTICLE

Heparanase Gene Expression Level In Egyptian Children With Acute Leukemia

Amira Ahmed Hammam M. D[1], Mohamed Aly El-Wakil M. D.[2] ; Samah Fathy Semary M. D[2]; Hesham Fathy Hedifa M. S. C.[3]

1. Department of Clinical and Chemical Pathology, Faculty of medicine, Beni suef university, Egypt.
2. Department of Clinical Oncology, Faculty of medicine, Beni suef university ,Egypt.
3. Department of Zoology (Molecular Biology), Faculty of Science, Al Azhar university, Egypt.

Manuscript Info

Manuscript History:

Received: 12 November 2013
Final Accepted: 23 December 2013
Published Online: January 2014

Key words:
Heparanase gene, Acute leukemia, AML, ALL, RTQ-PCR.

Abstract

Background: Heparanase is an endoglycosidase that degrades heparan sulfate, the main polysaccharide constituent of the extracellular matrix and basement membrane. The objective of this study was to show Heparanase gene expression pattern in acute leukemia patients and its role in disease severity and progression and in patients response to therapy.

Materials and Methods: We analyzed Heparanase gene expression level in 40 pediatric acute leukemia patients [20 acute myeloid leukemia (AML) and 20 acute lymphoblastic leukemia (ALL)] and 11 healthy controls using Real-Time Quantitative Reverse-Transcriptase Polymerase Chain Reaction (RTQ-PCR)

Results*:* Heparanase was expressed in 100% and 60% of AML and ALL respectively with levels significantly higher in patients than controls and in AML than ALL patients **(p<0.001)**. Heparanase levels were significantly correlated with blast percentage **(p=0.006)** , platelet count **(p=0.046)** and final status of the disease **(p<0.005)**.

Conclusion: We conclude that Heparanse gene is expressed in acute leukemia being higher in AML than ALL and controls. Patients with higher Heparanase gene showed poorer outcome. These findings suggest that Heparanase gene may be a novel significant therapeutic target for acute leukemia

Introduction

Acute leukemia is a heterogenous disease with numerous genetic abnormalities that define each subtype. Many of the known chromosomal translocations and mutations in leukemia target genes and pathways disrupt hematopoietic transcription factors and/or confer a proliferative and survival advantage to leukemia blasts [Mullighan CG et al., 2005].

Heparan sulfate proteoglycans (HSPGs) are ubiquitous macromolecules associated with the cell surface and extracellular matrix (ECM) of a wide range of tissues [Bernfield M et al.,1999]. The basic HSPG structure consists of a protein core to which several linear heparan sulfate (HS) chains are covalently O-linked. HS binds to and assembles extracellular matrix (ECM) proteins thus playing important roles in ECM integrity, barrier function, and cell- ECM interactions. The HS chains ensure that a wide variety of bioactive molecules bind to the cell surface and ECM and thereby function in the control of diverse normal and pathological processes [Sasisekharan R et al.,2002]. HSPGs not only provide a storage depot for heparin-binding molecules such as growth factors, chemokines and enzymes, but rather can decisively regulate their accessibility, function and mode of action [Iazzo RV,1998].

Recent discoveries indicated that HSPGs localized within the tumor microenvironment can be attacked by enzymes that alter proteoglycan structure resulting in dramatic effects on tumor growth and metastasis [Fjeldstad k and Kolset SO, 2005 and Sanderson RD et al, 2005].

Heparanase, an endoglycosidase, can specially cleave HS side chains from HSPGs and release a multitude of bioactive molecules. Then, the generated HS fragments and released bioactive mediators could facilitate tumor metastasis cooperatively. In addition , heparanase also exhibits non enzymatic activities, including cell adhesion and survival, upregulation of vascular endothelial growth factor(VEGF) and tissue factor, induction of signal transduction, and enhancement of certain HSPG from the tumor cell surface [Shuo D and Xiong-Zhi W, 2010 and Levy-Adam F et al, 2008].

A large body of evidence suggest that the expression of heparanase in the tumor closely relates with the potential for tumor invasion, angiogenesis and metastasis in most tumors examined [Parish CR et al,2001 and Vlodavsky I et al, 2008].

In this study, we analyzed the expression level of Heparanase in 40 acute leukemia patients [20 acute myeloid leukemia (AML) and 20 acute lymphoblastic leukemia (ALL) patients], together with 11 normal healthy age matched controls, using a Real-Time Quantitative Reverse-Transcriptase Polymerase Chain Reaction (RTQ-PCR) technique with the aim of showing the expression pattern of Heparanase gene in patients with acute leukemia and determining the role of this gene in disease severity and progression.

Materials and Methods

Patients and samples from patients

Peripheral blood (PB) samples and bone marrow (BM) aspiration samples from 40 Egyptian acute leukemia pediatric patients were collected at diagnosis and centrifuged to obtain PB and BM mononuclear cells (MNCS).

Patients were recruited from 2 places, Cairo university hospital and Beni Suef university hospital between March 2010 and March 2012.

The diagnosis of Acute leukemia was made based on the morphologic findings from Giemsa stained smears of bone marrow (BM) aspirates, cytochemical stains criteria such as negativity for myeloperoxidase (MPO) and sudan black B (SBB) in cases of ALL or their positivity in cases of AML and positivity of acid phosphatase for (T-ALL) and immunophenotyping criteria as CD10,CD19,CD20,CD22 for B-ALL,CD2,CD3,CD5,CD7 for T-ALL, and positivity of CD13 and CD33 for AML cases.

These patients diagnoses were distributed as follows: 20 acute myelogenous leukemia (AML) including the following subtypes of the French-American-British (FAB) classification (4 AML-M1; 10 AML-M2; 3 AML-M3; 1 AML-M5, 2 AML-M7), 20 acute lymphoblastic leukemia (ALL) from B ,T lineages or common ALL (14 B-lineage, 3 T-lineage and 3 common ALL).

Eleven age & sex matched individuals were included as controls .

Heparanase gene was analyzed using real time quantitative reverse transcriptase polymerase chain reaction (RTQ-PCR) to investigate a possible relation, association, or correlation with the clinical and laboratory features of patients at diagnosis, such as: sex, age, lineage (B or T), hemoglobin (HB), TLC, platelets count and BM blast cell infiltration; and patient outcome after treatment and follow up.

RNA isolation and real-time quantitative RT-PCR

Total RNA was extracted from peripheral blood mononuclear cells (MNCS) using a QIAamp RNA blood kit (Qiagen,Germany) according to the manufacture′s instructions. Total RNA (1µg) was used for a first strand complementary DNA (cDNA) synthesis using reverse transcription system (Promega, Madison,WI,USA) as described by the manufacturer and stored at -20°C till use. SYBR Green (Solis BioDyne, HOT FIREPOL EvaGreen) real- time PCRs for amplification of heparanase and the house keeping gene GAPDH were performed using ABI PRISM 7000 Sequence Detection Software (Applied Biosystems). The sequence of primers used for detection of the Heparanase gene cDNA were 5′-GTTCTAATGCTCAGTTGCTCCT-3′ and 5′-ACTGCGACCCATTGATGAAA-3′ and for GAPDH were 5′-GCACCGTCAAGGCTGAGAAC-3′ and 5′ TGGTGAAGACGCCAGTGGA-3′.

All reactions were performed in triplicate using 20µl samples containing 50ng cDNA. The reaction protocol used involved heating for 10 minutes at 95ºC, followed by 40 cycles of amplification (15 seconds at 94ºC and 1 minute at 60ºC).

The expression levels of Heparanase gene in tested samples were expressed in the form of CT (cycle threshold) level then Normalized copy number (Relative quantitation) was calculated using the ΔΔ CT equation. A negative control without template was included in each experiment.

Expression level of Heparanase gene was correlated with the clinical features of the studied patients at diagnosis including: age, sex, TLC, hemoglobin, platelets, lineage, etc.

Statistical Methods

Data was analyzed using IBM SPSS advanced statistics version 20 (SPSS Inc., Chicago, IL). Numerical data of scores were expressed as mean and standard deviation or median and range as appropriate. Qualitative data were expressed as frequency and percentage. Chi-square test (Fisher's exact test) was used to examine the relation between qualitative variables. For not normally distributed quantitative data, comparison between two groups was done using Mann-Whitney test (non-parametric t-test). Comparison between 3 groups was done using Kruskal-Wallis test (non-parametric ANOVA) then post-Hoc "Schefe test" on rank of variables was used for pair-wise comparison. Spearman-rho method was used to test correlation between numerical variables. Survival analysis was done using Kaplan-Meier method and comparison between two survival curves was done using log-rank test. Odds ratio (OR) with it 95% confidence interval (CI) were used for risk estimation. The Receiver Operating Characteristic (ROC) curve was used for prediction of cut off values. Kappa test was used to evaluate agreement between two diagnostic methods. A p-value < 0.05 was considered significant.

Results

The present study was conducted on 40 denovo pediatric acute leukemia patients (20 AML and 20 ALL), clinical characteristics of which are presented in table (1). Eleven normal age and sex matched normal volunteers were studied as a control group.

They were 23 male patients (10 AML&13 ALL) and 17 female patients (10 AML &7 ALL), while controls were 6 males and 5 females.

Concerning AML Patients

They were 20 AML patients; 10 males (50%) and 10 females (50%), their ages ranged from (0.8-17 years) with mean 8.5 ±5.0 years and median 7.5 years. The cases were : 4 cases M1(20%), 10 M2 (50%), 3 M3(15%), 1 M5(5%), 2 M7(10%). The mean total leucocytic count (TLC) at diagnosis was 36.1 ± 34.1x10^3 with range (1.2-140x10^3) and median 29x10^3, mean hemoglobin was 7.1±2.4 gm/dl with range (3.3-13.4 gm/dl) and median 6.9 gm/dl, mean platelet count was 25.7±17.9x10^3 with range (2-70x10^3) and median 20x10^3, mean peripheral blood blasts was 48.3±32.5% with range (10-84%) and median 50.5%, the mean bone marrow blasts was 52.8±26.8% with range (15-95%) and median 55%. 20/20 ALL patients (100%) had no CSF involvement. 4/20 (20%) of AML patients had no organomegaly while 4/20(20%) had splenomegaly, 3/20(15%) had hepatomegaly, 9/20(45%) had hepatosplenomegaly and 6/20(30%) patients had no enlarged lymph nodes while 14/20(70%) had enlarged lymph nodes. After induction chemotherapy 18/20(90%) of AML patients entered in complete remission (CR) while 2/20(10%) showed no CR.

Concerning ALL Patients

They were 20 ALL patients; 13 males (65%) and 7 females (35%), their ages ranged from (0.7-15 years) with mean 7.2 ±4.6 years and median 7.0 years. The cases were: 13 cases pre B (65%), 1 pro B (5%), 3 C ALL (15%), 3 TALL (15%). The mean total leucocytic count (TLC) at diagnosis was 82.1 ± 125.2x10^3 with range (1.7-452x10^3) and median 31.5x10^3, mean hemoglobin was 8.2±2.4 gm/dl with range (4.3-14 gm/dl) and median 7.7 gm/dl, mean platelet count was 51.1±39.5x10^3 with range (7-152x10^3) and median 39x10^3, mean peripheral blood blasts was 36.6±30.2% with range (10-97%) and median 24.5%, the mean bone marrow blasts was 74.3±27.7% with range (8-98%) and median 87%. 17/20 ALL patients (85%) had no CSF involvement while 3/20(15%) had CSF involvement. 3/20 (15%) of ALL patients had no organomegaly, while 5/20 (25%) had splenomegaly, 1/20 (5%) had hepatomegaly, 10/20 (50%) had hepatosplenomegaly and 1/20(5%) had mesenteric or splenic hilar lymph nodes. 3/20(15%) ALL patients had no enlarged lymph nodes while 17/20(85%) had enlarged lymph nodes. After induction chemotherapy 19/20(95%) of ALL patients entered in complete remission (CR) while 1/20(5%) showed no CR.

Comparative studies

Comparing AML and ALL patients as regards their clinical and laboratory data showed no statistical significance for TLC, hemoglobin and peripheral blood blasts p value being (0.659,0.114 and 0.46) respectively, but was of statistically significant difference for platelets **(p=0.046)** and bone marrow blasts **(p=0.006)**

Comparing the 3 groups as regards the Heparanase gene level there was high statistical significant difference **(p<0.001)** being maximum in AML and minimum in controls, with mean Relative quantitation (RQ) level 2,336.2± 10,405.2 in AML, median 8.0 and range (3.1-46,543.0), while mean RQ in ALL was 1.7±1.0 ,median 1.7 and range (0.1-3.1) and in controls mean was 0.8±0.3, median 0.8 and range (0.4-1.4) (Fig 1&table 2).

Comparison between each 2 groups as regards heparanase level was of high statistically significant difference, p value being **(p<0.001)** when comparing AML/ALL and AML/controls and **(p=0.035)** when comparing ALL/controls.

Cut off value for heparanase gene was calculated using Roc curve and was found to be **1.413** with 80% sensitivity and 100% specificity. According to this cut off level, 20/20 (100%) AML cases were heparanase positive, 12/20(60%) ALL cases were heparanase positive and 8/20 ALL patients were negative, while all controls (100%) were negative. This was of high statistical significance **(p<0.001)**.

Follow up of the whole patients group showed that post induction chemotherapy 37/40(92.5%) entered CR while 3/40 (7.5%) showed no CR, the event of these 37 patients after follow up of showed that 34/37 continued to be in CR while 3/37 relapsed. As for the final state of the disease (FSD) 33/40 (82.5%) of patients were in CR and 7/40(17.5%) had either stationary or progressive disease (PD) and for the state of the patient (SD); 33/40(82.5%) were alive free (AF), 3/40(7.5%) were alive diseased (AD) and 4/40 were dead. The overall survival time of the whole group mean was 20.48±8.37 month with median 21.12 and range (0-34.9 month), while the disease free survival (DFS) time mean was19.28±7.22 month, median 19.34 month and range (1.61-34.28) month. Survival analysis of patients till the end of the study (34.9 month) was **89.73%** (no median survival because more than half of the patients were still alive till the end of the study).

Comparing the OS of AML/ALL there was no statistically significant difference (p=0.2916), while comparing the DFS of AML/ALL was of statistical significant difference **(0.0312) (figure 2).** Comparing the OS and DFS of the whole group as regards heparanase gene expression showed no statistically significant difference with (p=0.2968 &0.2739) respectively, this may be because only 4 patients had different event (death). Also analyses of OS and DFS in each group and its relation to heparanase gene showed no statistical significance (p=0.4142 and 0.1) respectively (Table 3)

Comparing the final status of the disease (CR/ stationary or PD) as regards the heparanase gene level (RQ), showed high statistical significant difference **(p<0.005)** with the level being higher in patients with stationary/PD**.** There were 7/40 (17.5%) patients in PD with heparanase gene level (RQ) mean 6,662.40653±7,436.4754, range (1.46-46,543.02) and median 17.19 and 33/40(82.5%) patients in CR with heparanase gene level (RQ) mean 3.67663± 2.8543, range (9.381189- 0.096973) and median 1.08.

Correlation studies

There was no significant correlation between all group and heparanase gene level as regards age, TLC, hemoglobin, platelets and peripheral blood blasts (p=0.353; 0.704; 0.844; 0.54 and 0.097) respectively, while there was significant negative correlation on comparing bone marrow blast% and heparanase gene level **(r=-0.408 and p=0.09).**

Correlation between heparanase +ve and heparanase –ve cases within AML and ALL groups as regards clinical and laboratory data including age, sex , TLC, hemoglobin, platelets, bone marrow and peripheral blood blasts, CSF affection, organomegaly and lymphadenopathy; and as regards the response to therapy (CR and no CR) there was no statistically significant difference.

Table (1): Clinical and Laboratory data of the patients and its relation to Heparanase gene

	AML n=20	**ALL n=20**	**p-value**
Age(years)			
mean±SD	8.5±5	7.2±4.6	0.589
median	7.5	7.0	
range	0.8-17.0	0.7-15.0	
Sex (n,%)			
Male	10 (50%)	13 (65%)	0.623
Female	10 (50%)	7 (35%)	
TLCX10^3			
mean±SD	36.1±34.1	82.1±125.2	0.659
median	29.0	31.5	
range	1.2-140.0	1.7-452.0	
Haemoglobin(gm/dl)			
mean±SD	7.1±2.4	8.2±2.4	0.114
median	6.9	7.7	
range	3.3-13.4	4.3-14.0	
PlateletsX 10^3			
mean±SD	25.7±17.9	51.1±39.5	***0.046**

median range	20.0 2-70	39.0 7.0-152.0	
P.B. Blast (%) mean±SD median range	 48.3±32.5 20.0 2.0-70.0	 36.6±30.2 24.5 10-97	 0.494
B.M Blast (%) mean±SD median range	 52.8±26.8 55.0 15-95	 74.3±27.7 87.0 8.0-98	 ***0.006**
CSF (%) Negative positive	 20(%) 0(0%)	 17(85%) 3(15%)	 _____
Organomegaly (n%) Negative Positive	 4(20%) 16(80%)	 3(15%) 17(85%)	 1.000
Lymphadenopathy Negative Positive	 6(30%) 14(70%)	 3(15%) 17(85%)	 0.256
Response CR (n %) No CR (n %)	 18 (90%) 2 (10%)	 19(95%) 1(5%)	 _____

Table (2): Heparanase gene expression rates and levels in the 3 studied groups

Heparanase gene(cut off 1.413)	AML n=20	ALL n=20	Control n=11	P-Value
Rate(%) Negative Positive	 0/20(0%) 20/20(100%)	 8/20(40%) 12/20(60%)	 11/11(100%) 0/11(0%)	 ***P1<0.001** ***P2=0.003**
Level (RQ) mean±SD median range	 2336.2±10405.2 8.0 3.1-46543.0	 1.7±1.0 1.7 0.1-3.1	 0.8±0.3 0.8 0.4-1.4	 ***P1<0.001** ***P2<0.001** ***P3=0.035** ***P4<0.001**

P1: AML/ALL/Controls P2: AML/ALL P3: ALL/Controls P4: AML/Controls

Table (3): Relationship between patient outcome and heparanase gene expression

Group	Overall survival (event) n=40	Disease free survival (event) n=37	p-value
Acute leukemia Heparanase -ve Heparanase +ve	 8(0) 32(4)	 8(0) 29(4)	 0.2968 0.2739
AML Heparanase -ve Heparanase +ve	 0(0) 20(3)	 0(0) 18(4)	 -------- ---------

ALL			
Heparanase -ve	8(0)	8(0)	0.4142
Heparanase +ve	12(1)	11(0)	0

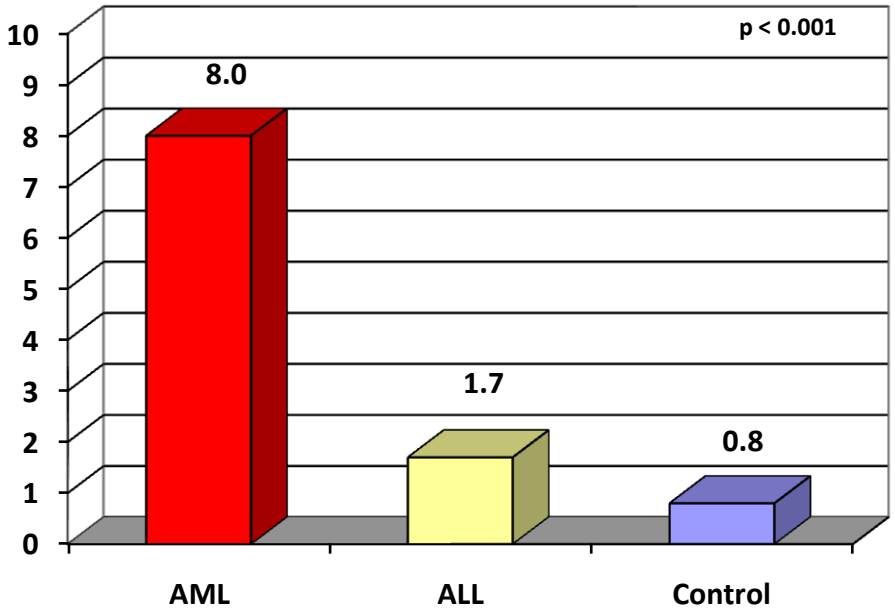

Figure 1: Median heparanase level (RQ) in AML, ALL and control groups

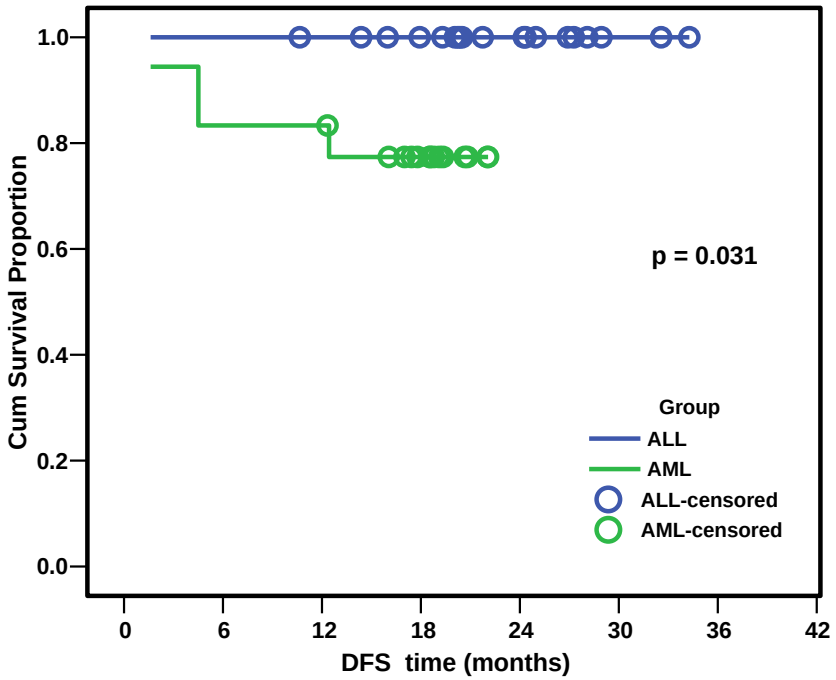

Figure 2: Disease free survival of the AML and ALL groups

Discussion

Although the study of enzymes that cleave heparan sulfate is in its infancy, recent finding imply that heparanases play a critical role in promoting tumor growth and metastasis. In humans, heparanase-1 (heparanase) appears to be the dominant, if not the only heparanase that can cleave extracellular heparan sulfate [Vlodavsky I et al ,1999; Kussie PH et al,1999; Hulett MD et al,1999; Toyoshima M and Nakajima M,1999]. Although other heparanases are likely present in many cells, their activity appears to be localized to intracellular compartments [McKenzie E et al ,2000; Bame KJ et al, 2002].

Heparanase expression is rare in normal tissues, but becomes evident in many human tumors where it significantly increases both the angiogenic and metastatic potential of tumor cells [Vloddavsky I et al, 2002]. Elevated heparanase expression in humans has been correlated with advanced progression and metastasis of tumors of the breast [Maxhimer JB et al, 2002], colon [Freidmann Y et al, 2000], ovary [Ginth S et al,2001], bladder [Gohji K et al, 2001], pancreas [Kolioponas A et al,2001] and acute myeloid leukemia [Bitan M et al, 2002].

Another example of these genes is neuropilin-1(NRP-1). The relation between NRP-1 and acute leukemia is not really surprising due to the growing evidence that solid tumors as well as hematological malignancies are dependent on neovascularization

and since NRP-1 is the receptor of VEGF, it is highly suggested to be implied in the progression of hematological malignancies [Younan S et al,2012].

The mechanism of heparanase function in tumors is under intense investigations and it appears that this enzyme may have multiple effects. For example, degradation of heparan sulfate likely facilitates tumor cell motility and budding of new blood vessels, an event that requires remodeling of the heparan sulfate-rich basement membrane.

Heparan sulfate proteoglycans within the tumor microenvironment can serve as a reservoir of heparin-binding growth factors and chemokines, and the activity of heparanase may act to release these factors for use by the tumor cells. Moreover it is important to note that active heparanase does not completely digest the heparan sulfate chains it attacks; rather it selectively cleaves the glycoside bonds of heparan sulfate chains at only a few specific sites, producing fragments that are only 10 to 20 sugar residues long [Bitan M et al, 2002 and Vlodavsky I and Friedmann Y,2001]. These fragments generated by heparanase or by enzymes that cleave heparan sulfate in a manner similar to that of heparanase have been shown to enhance the ability of heparan sulfate to potentiate the activity of bound growth factors [Kato M et al,1998 and Liu D,2002]. Thus, degradation of heparan sulfate surrounding the tumor may remove physical barriers and enhance signaling events that spark metastasis.

The present study revealed that Heparanase gene is significantly associated with acute leukemia and that its level correlates with disease severity and progression. This was demonstrated by the analysis of Heparanase gene expression level in 40 pediatric acute leukemia patients and eleven controls using a Real-Time Quantitative Reverse-Transcriptase Polymerase Chain Reaction (RTQ-PCR) technique and correlating this level with different clinical and laboratory features among different groups; where Heparanase was found to be expressed in a high proportion of patients compared to controls [the expression rate is 100% and 60% of AML and ALL patients respectively] compared to controls and with a significantly higher level in AML than ALL and controls. Also, higher Heparanase levels were significantly correlated with blast percentage and final state of disease. Interestingly enough, we detected a significantly higher heparanase expression level in patients with final status of disease stationary/PD compared to those with CR as final status.

O Ostrovsky, et al (2007) investigated heparanase gene polymorphisms in patients acute myeloid leukemia (AML), myelodysplastic syndrome (MDS), acute lymphoblastic leukemia (ALL), chronic myeloid leukemia (CML), Hodgkin's disease (HD) and multiple myeloma (MM).

They found Significant correlation between rs11099592 and rs6535455 heparanase gene (HPSE) single nucleotide polymorphisms (SNPs) and ALL. Genotype frequency comparisons revealed a significant association with rs4693602 in MM patients and rs4364254 in AML patients. They examined heparanase gene mRNA expression by real-time RT-PCR and indicated a significant low HPSE gene expression level in ALL patients and a high expression level in MM and AML patients, compared to healthy controls. Moreover, statistically significant correlation was found between heparanase mRNA expression level and three HPSE gene SNPs (rs4693608, rs11099592 and rs4364254) among healthy individuals. They concluded that these data suggest that

certain HPSE gene SNPs may contribute to basal heparanase gene expression and that alterations in this gene are an important determinant in the pathogenesis of ALL, AML and MM. This was in accordance to our work concerning AML, although we detected low levels of heparanase m RNA in ALL patients as well as normal control samples which they didn't detect.

Bitan M et al (2002) evaluated heparanase expression in leukocytes isolated from peripheral blood of 71 patients with myeloid and lymphoid leukemias, or non-Hodgkins lymphoma. Analysis was performed at two levels: heparanase RNA was determined by reverse transcriptase polymerase chain reaction, and heparanase protein was evaluated by immunocytochemistry and flowcytometry. They found that in eight peripheral blood samples from normal donors, heparanase RNA was detected, and protein was found within the cytoplasm of granulocytes. In mononuclear cells derived from various leukemias, heparanase RNA was expressed in 14 of 15 acute myeloid leukemia (AML) samples. In contrast, cells derived from all 33 chronic lymphoblastic leukemia, all 7 non-Hodgkins lymphoma, 7 of 8 chronic myeloid leukemia, and 6 of 8 acute lymphoblastic leukemia patients showed no detectable expression of the heparanase RNA. They also revelead that heparanase protein was detected primarily within the cytoplasm of AML cells, indicating that the enzyme is produced and stored within the cytoplasm of myeloid cells, with limited expression on the cell surface. This was in agreement with our results concerning AML but we detected low levels of heparanase mRNA in ALL as well as in normal controls.

Thomas K et al (2003) analyzed the activity, expression, and function of heparanase in myeloma patients. They analyzed heparanase activity in the plasma isolated from bone marrow biopsies of 100 patients and revealed 86 positive for heparanase activity and 14 negative. In contrast to the bone marrow plasma, levels of heparanase activity in peripheral blood plasma of 29 myeloma patients were found to be either negative or low, they suggested that in multiple myeloma, heparanase functions in the local microenvironment of the bone marrow and its activity is not significantly elevated systemically. Immunohistochemistry was performed and revealed that patients with high levels of heparanase activity often have tumor cells with intense staining for the enzyme. Interestingly, they noted a marked heterogeneity among tumor cells, with clusters of heavily stained cells surrounded by cells with weak or negative staining for heparanase. They analyzed microvessel density and revealed a strikingly higher concentration of vessels in patients with high heparanase activity as compared with patients negative for heparanase activity. When they implanted human myeloma cells transfected with the cDNA for heparanase in severe combined immunodeficient (SCID) mice, the resulting tumors exhibited a significantly higher microvessel density than did tumors established with control cells. Thus they concluded that expression of heparanase appears to play a direct role in enhancing microvessel density in myeloma cells which is the same hypothesis that we suggest.

In conclusion, our findings suggest that Heparanase gene is significantly associated with pediatric acute leukemia and that its level might serve as an indicator for disease progression. Heparanase can be considered as a potential target for antileukemic treatment strategies in acute leukemia.

References

1) Bame KJ, Venkatesan I, Dehdashti J, McFarlane J, Burfeind R. (2002): Characterization of a novel intracellular heparanase that has a FERM domain. Biochem J;364:265-274
2) Bernfield M, Gotte M, Park PW, et al. (1999): Functions of cell surface heparin sulfate proteoglycans. Annu Rev Biochem ; 68:729-77
3) Bitan M, Polliack A, Zecchina G, et al. (2002): Heparanase expression in human leukemias is restricted to acute myeloid leukemia. Exp Hemato.;30;34-41
4) Fjeldstad k, Kolset SO (2005): Decreasing the metastatic potential in cancers targeting the heparan sulfate proteoglycans. Curr Drug Targets ; 6:665-682
5) Freidmann Y, Vlodavsky I, Aingorn H, et al. (2000): Expression of heparanase in normal dysplastic and neoplastic human colonic mucosa and stroma: evidence for its role in colonic tumorigenesis. Am J Path ; 157: 1167-1175
6) Ginth S, Menczer J, Friedmann Y, et al. (2001): Expression of heparanase .Mdm2 and erbB2 in ovarian cancer. Int J Oncol; 18:1133-1144
7) Gohji K, Okamoto M, Kitazawa S, et al.(2001): Heparanase protein and gene expression in bladder cancer.J Urol; 166:1286-1290
8) Hulett MD, Freeman C, Hamdorf BJ, Baker RT, Harris MJ, Parish CR.(1999): Cloning of mammalian heparanase, an important enzyme in tumor invasion and metastasis. Nat Med; 5:803-809
9) Iazzo RV. Matrix proteoglycans(1998): from molecular design to cellular function. Annu Rev Biochem; 67: 609-52
10) Kato M, Wang H, Kainulainen V, et al. (1998): physiological degradation converts the soluble syndecan-1 ectodomain from an inhibitor to a potent activator of FGF-2. Nat Med; 4:691-697
11) Kolioponas A, Friess H, Kleeff J, et al. (2001): Heparanase expression in primary and metastatic pancreatic cancer. Cancer Res;61:4655-4659

12) Kussie PH, Hulmes JD, Ludwig DL, et al. (1999): Cloning and functional expression of a human heparanase gene. Biochem Biophys Res Commun ;261:183-187
13) Levy-Adam F, Feld S, Suss-Toby E, Vlodavsky I, Ilan N. (2008): Heparanase facilitates cell adhesion and spreading by clustering of cell surface heparin sulfate proteoglycans. Plos One; 3:e2319
14) Liu D, Shriver Z, Venkataraman G, El Shabrawi Y, Sasisekharan R. (2002): Tumor cell surface heparin sulfate as cryptic promoters or inhibitors of tumor growth and metastasis. Proc Natl Acad Sci U S A ; 99:568-573
15) Maxhimer JB, Quiros RM, Stewart R, et al. (2002): Heparanase-1 expression is associated with the metastatic potential of breast cancer. Surgery ;132:326-333
16) McKenzie E, Tyson K, Stamps A. et al.(2000): Cloning and expression profiling of Hpa2, a novel mammalian heparanase family member. Biochem Biophys Res Commun ;276:1170-1177
17) Mullighan CG, Flotho C and Downing JR. (2005): Genomic assessment of pediatric acute leukemia. Cancer j ;11:268-282
18) Ostrovsky O, Korostieshevsky M, Levite I, et al.(2007): Association of heparanase gene (HPSE) single nucleotide polymorphisms with hematological malignancies. Leukemia;21:2296-2303
19) Parish CR, Freeman C, Hulett MD. (2001): Heparanase, a key enzyme involved in cell invasion. Biochem Biophys Acta ; 1471:M99-M108
20) Sanderson RD, Yang Y, Kelly T, MacLeod V, Dai Y and Theus A(2005): .Enzymatic remodeling of heparin sulfate proteoglycans within the tumor microenvironment, growth regulation and the prospect of new cancer therapies. J Cell Biochem;96:897-905
21) Sasisekharan R, Shriver Z, Venkataraman G, Narayanasami U. (2002): Roles of heparin-sulphate glycosaminoglycans in cancer. Nat Rev Cancer ; 2:521-8
22) Shuo D and Xiong-Zhi W. (2010): Heparanase and hepatocellular carcinoma: Promoter or inhibitor? World J Gastroenterol; January 21; 16(3):306-3011
23) Thomas K, Hua-Quan M, Yang Y, et al. (2003): High heparanase activity in multiple myeloma is associated with elevated microvessel density. Cancer Reasearch ;63: 8749-8756
24) Toyoshima M and Nakajima M. (1999): Human heparanase purification, characterization, cloning, and expression . J Biol Chem; 274: 24153-24160
25) Vlodavsky I, Friedmann Y, Elkin M, et al.(1999): Mammalian heparanase gene cloning, expression and function in tumor progression and metastasis. Nat Med ; 5:793-802
26) Vlodavsky I and Friedmann Y. (2001): Molecular properties and involvement of heparanase in cancer metastasis and angiogenesis. J Clin Invest ;108: 341-347
27) Vloddavsky I, Goldshmidt O, Zcharia E, et al.(2002): Mammalian heparanase involvement in cancer metastasis, angiogenesis and normal development. Semin Cancer Biol; 12:121-129
28) Vlodavsky I, Elkin M, Abboud-Jarrous G, et al. (2008): Heparanase, one molecule with multiple functions in cancer progression. Connect Tissue Res ; 49:207-210
29) Younan S, Elhoseiny S, Hammam A, et al. (2012): Role of Neuropilin-1 and its expression in Egyptian Acute Myeloid and Acute Lymphoid Leukemia patients. Leukemia Research ; 36(2): 169– 173.