BNIP3L in myelodysplastic syndromes and acute myeloid leukemia: impact on disease outcome and cellular response to decitabine

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doi:10.3324/haematol.2016.142521

Supplementary Material

BNIP3L expression in myelodysplastic syndromes and acute myeloid leukemia: impact on disease outcome and cellular response to decitabine

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Supplementary Table S1. Patient characteristics

Patients	Number (gene expression analysis)	Number[1] (methylation analysis)
MDS	**65**	**45**
Gender		
Male/Female	38/27	18/27
Age (years), median (range):	70 (16-87)	70 (16-85)
WHO 2008 classification		
RA/RARS/del(5q)/RCMD	3/4/1/41	1/2/1/29
RAEB-1/RAEB-2	9/7	8/4
IPSS		
Low risk/intermediate-1	21/33	14/23
Intermediate-2/high risk	5/3	4/1
Not available	3	3
Cytogenetic risk[2]		
Good	52	35
Intermediate	8	3
Poor	2	4
No growth	3	3
AML	**74**	**45**
de novo AML/AML-MRC	61/13	39/6
Gender		
Male/Female	39/35	22/23
Age (years), median (range):	60 (18-90)	61 (23-93)
BM blasts (%), median (range)	68 (20-98)	69 (20-98)
Cytogenetic risk[3]		
Good	8	3
Intermediate/poor	43/13	28/5
No growth	10	9

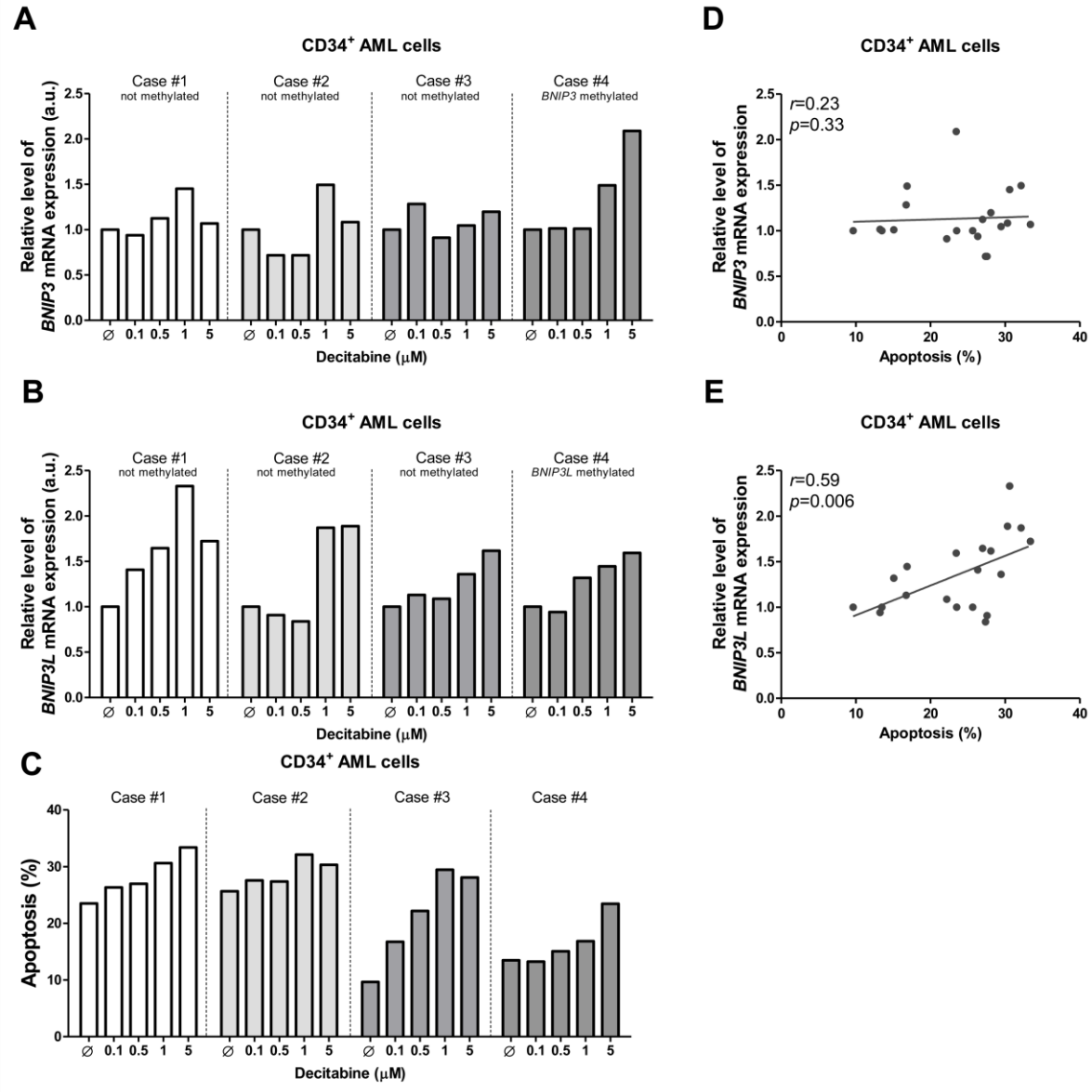
Abbreviations: MDS, myelodysplastic syndromes; WHO, World Health Organization; RA, refractory anemia; RARS, refractory anemia with ringed sideroblasts; del(5q), MDS with isolated del(5q);

RCMD, refractory cytopenia with multilineage dysplasia; RAEB-1, refractory anemia with excess blast-1; RAEB-2, refractory anemia with excess blast-2; IPSS, International Prognostic Scoring System; BM, bone marrow; AML, acute myeloid leukemia; AML-MRC, acute myeloid leukemia with myelodysplasia-related changes.

[1]Among the patients included in the methylation analysis, 5 MDS and 16 AML patients were not evaluated for gene expression, whereas 40 MDS and 29 AML patients were included in both gene expression and methylation cohorts.

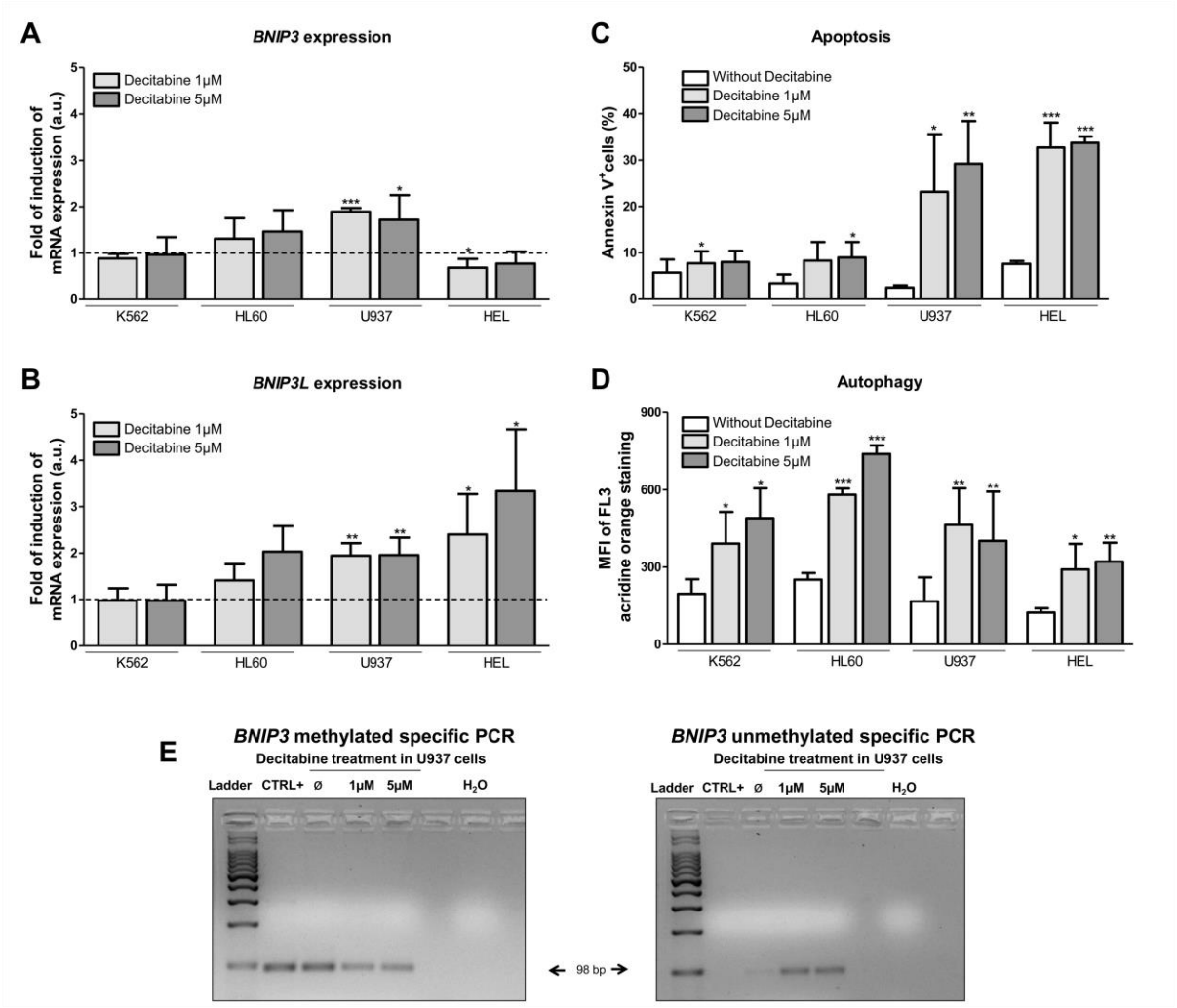
[2]Cytogenetic risk for MDS was defined according to IPSS.[1]

[3]Cytogenetic risk for AML was defined according to Grimwade et al.[2]



Supplementary Figure S1. *BNIP3* and *BNIP3L* expression and apoptosis of primary cells from AML patients treated with decitabine. $CD34^+$ bone marrow cells from four AML patients were treated with decitabine (0.1 µM, 0.5 µM, 1µM or 5µM) for 48 hours. Gene expression was evaluated by quantitative PCR and apoptosis was measured by flow cytometry using Annexin-V/PI staining. Relative expression of *BNIP3* (A) and *BNIP3L* (B).

Transcript levels were normalized to those of untreated cells and HPRT was used as a housekeeping gene. Data are expressed in arbitrary units (a.u.). (C) Apoptosis was measured by the percentage of the Annexin V-positive/PI-negative cells. Correlation between *BNIP3* (D) or *BNIP3L* (E) and apoptosis induced by decitabine treatment. Spearman correlation test, *p* and *r* values are indicated in the graph.



Supplementary Figure S2. *BNIP3* and *BNIP3L* expression, apoptosis and autophagy of myeloid leukemia cell lines treated with decitabine. Relative expression of *BNIP3* **(A)** and *BNIP3L* **(B)** in K562, HL60, U937 and HEL cells treated with 1µM or 5µM decitabine for 48 hours. Transcript levels were normalized to those of untreated cells (dotted line) and HPRT was used as a housekeeping gene. Data are reported as the mean ± SD (n=4) and are expressed in arbitrary units (a.u.). **(C)** Apoptosis and (**D)** autophagy induced by decitabine treatment in K562, HL60, U937 and HEL cells. Data are reported as the mean ± SD (n=4). **p*<0.05, ***p*<0.01,

***p<0.001; Student's t-test. **(E)** Analysis of BNIP3 methylation in U937 cells treated or not with decitabine (1µM or 5µM) for 48 hours. Sample from a universal methylated human DNA was used as the positive control.

References

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