Oncogenic splicing of Apoptosis-stimulating of p53 protein 2 (ASPP2) promotes leukemogenesis and therapy resistance in acute myeloid leukemia (AML) *in vivo*

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BACKGROUND

Despite remarkable progress in the last decades, the majority of patients diagnosed with AML face a poor prognosis. Prognostic scores have been established to guide therapy for patients that are intensively treated. Nevertheless, rates of refractory or relapsing (R/R) disease remain high and even in the favorable risk situation patients face a considerable risk of relapse. Additional or better markers are needed to predict therapy outcome and guide treatment decisions. iASPP is an oncogene and an evolutionarily conserved inhibitor of p53-mediated apoptosis and has been shown to have pro-proliferative and chemoresistant properties. High levels of iASPP are associated with poor prognosis in several cancers. We here assessed iASPP expression, function and its predictive value in AML.

METHODS

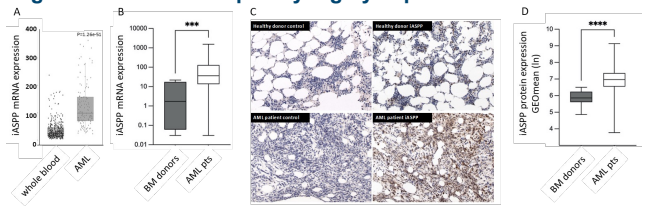
A large RNAseq leukemia cohort and myeloid tissue deriving from three repositories (TCGA, TARGET and GTEx, n>50.000) was used to assess for *iASPP* expression patterns in AML. In addition, an independent random validation cohort of newly diagnosed patients with AML (n=99) and a bone marrow donor cohort (n=31) and two clinical trial cohorts of intensively treated patients (HOVON study group cohorts) with follow-up data were further studied to confirm iASSP expression and correlate with survival data. We demonstrate that *iASPP* is frequently overexpressed in AML in all tested patient cohorts ($p \leq 0.001$). Higher expression levels were confirmed in secondary AML (p=0.015) and R/R AML compared to de novo AML (p=0.04). Analysis of the clinical validation cohorts reveal that high iASPP expression levels associate with poorer survival rates. Notably, the predictive role of *iASPP* is independent of, and adds information to, the European LeukemiaNET (ELN) risk classification. In line, silencing of iASPP in a MOLM14 model attenuated proliferation rates of leukemic blasts (p=0.028 at 48hrs) and rendered cells more susceptible to cytotoxic therapy (eg. daunorubicin 5nm: apoptosis rate at 24h: 40% in EV vs. 65% in KD cells). Consequently, a xenograft mouse model demonstrated a significant delay in disease onset and tumor burden in iASPP-silenced strains.

CONCLUSIONS

Together, we demonstrate that iASPP is frequently highly expressed and functionally active in AML, rendering leukemic blasts to a more aggressive phenotype and that high *iASPP* expression predicts for an unfavorable outcome in patients with AML.

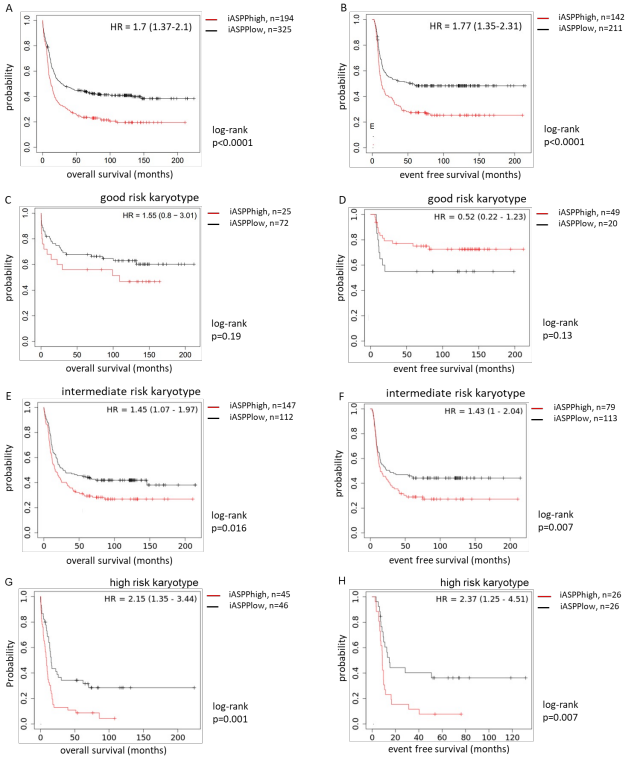
RESULTS

Figure 1. iASPP is frequently highly expressed in AML



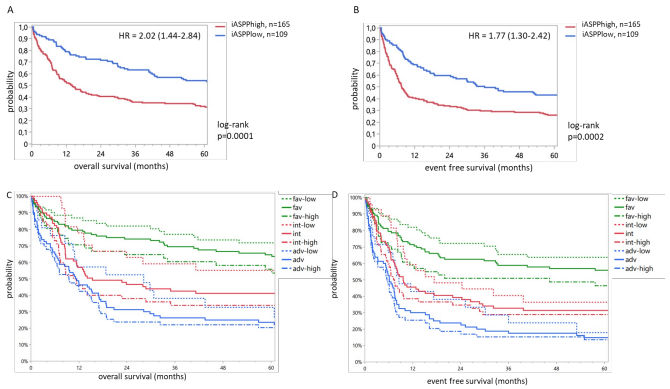
(A) Relative iASPP mRNA expression levels of a large RNAseq dataset including n=296 pts with AML compared to physiologic whole blood samples, n=670. (B) Relative iASPP mRNA expression levels of 43 AML patients compared to samples from healthy bone marrow (BM) donors (n=11). GAPDH served as a housekeeping gene. (C) Immunohistochemistry stains (40x) of iASPP in bone marrow of a healthy donor and a patient with AML. (D) Relative iASPP protein expression levels in 73 patients with newly diagnosed AML compared to mononuclear cells from 31 healthy bone marrow (BM) donors as detected by immunostaining in a flow cytometer approach. Isotype IgG controls served as basal levels.

Figure 2. iASPP expression in AML correlates with clinical outcome



iASPP and survival in AML. (A, C, E, G) OS and (B, D, F, H) EFS according to iASPP expression and risk category acc. to karyotype in a transcriptomic dataset (GSE6891).

Figure 3. iASPP and survival in a patient cohort treated in the HOVON102 trial

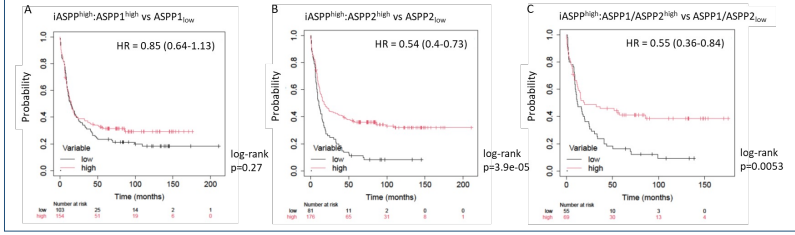


(A) OS and (B) EFS according to iASPP expression level. (C) OS, (D) EFS hazard ratios according to iASPP expression levels and ELN 2017 risk score (favorable/intermediate/adverse risk +/- iASPPlow or iASPPhigh). (E) 1- and 2-year overall survival rates acc. to iASPP expression. n=274

E

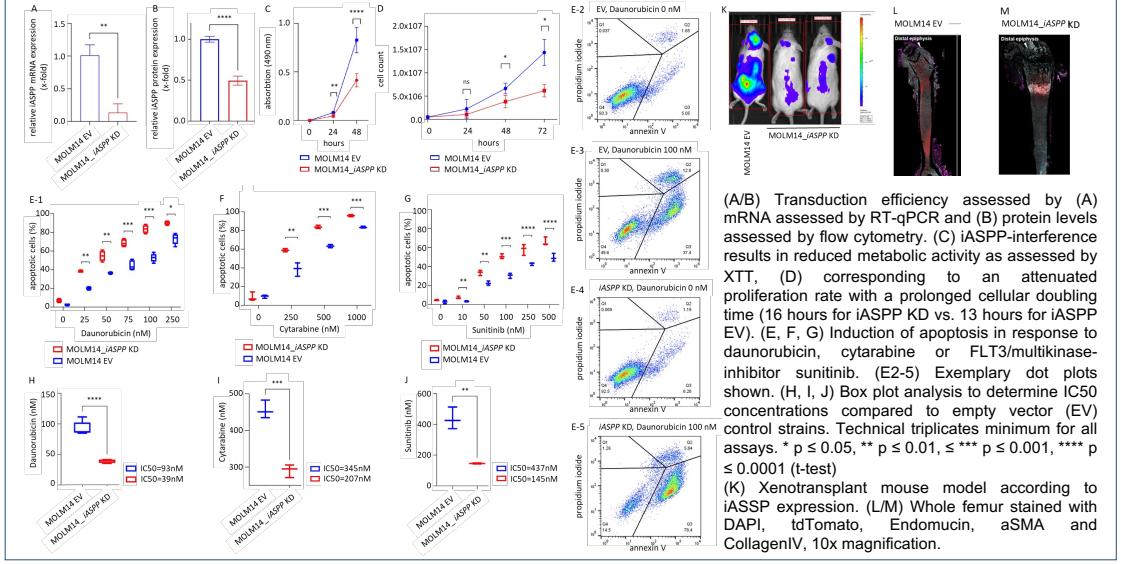
	1- and 2- year Overall Survival		
ELN 2017		iASPP high (95% CI)	iASPP low (95% CI)
Favorable	1-year	70.6%	86.9%
	2-year	64.7%	82.0%
Intermediate	1-year	45.9%	77.8%
	2-year	37.9%	63.0%
Adverse	1-year	42.4%	57.1%
	2-year	23.4%	52.4%

Figure 4. Overall survival according to ASPP1 and/or ASPP2 coexpression with iASPP



Analysis of a iASPP high-expressor cohort (transcriptomic dataset (GSE6891)) reveals that coexpression (variable) of ASPP2 (p=0.00004), ASPP1+ASPP2 (p=0.0053), but not ASPP1 (p=0.27) has a beneficial effect with regard to OS (i.e. compensates the adverse effect of iASPP in this cohort).

Figure 5. Functional analysis of iASPP in a MOLM14 interference cell line model.



(A/B) Transduction efficiency assessed by (A) mRNA assessed by RT-qPCR and (B) protein levels assessed by flow cytometry. (C) iASPP-interference results in reduced metabolic activity as assessed by XTT, (D) corresponding to an attenuated proliferation rate with a prolonged cellular doubling time (16 hours for iASPP KD vs. 13 hours for iASPP EV). (E, F, G) Induction of apoptosis in response to daunorubicin, cytarabine or FLT3/multikinase-inhibitor sunitinib. (E2-5) Exemplary dot plots shown. (H, I, J) Box plot analysis to determine IC50 concentrations compared to empty vector (EV) control strains. Technical triplicates minimum for all assays. * $p \leq 0.05$, ** $p \leq 0.01$, ≤ *** $p \leq 0.001$, **** $p \leq 0.0001$ (t-test)

(K) Xenotransplant mouse model according to iASSP expression. (L/M) Whole femur stained with DAPI, tdTomato, Endomucin, aSMA and CollagenIV, 10x magnification.

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