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Isocitrate dehydrogenase 1 (IDH1) mutation-specific twelve microRNA signatures as prognostic biomarkers in acute myeloid leukemia

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BACKGROUNDS

- **Acute myeloid leukemia (AML)** is known to be very **heterogeneous** in both its characteristics and treatment outcomes
- To select the appropriate therapy protocol, it is important to **categorize patients into discrete risk groups**.
- Depending on cytogenetic status, AML pts divided into

       CF-AML (cytogenetically favorable-risk AML)

       CN-AML (cytogenetically normal-risk AML)

       CP-AML (cytogenetically poor-risk AML)

- Recently, it was shown that along with cytogenetic abnormalities, **mutational analysis** in AML have become important prognostic marker that can be used to improve prediction of patient outcome and to direct therapy in AML.
- For example, cytogenetically intermediate-/normal-risk AML (CN-AML) patients can be reclassified as favorable-, intermediate- or poor-risk based on the mutational status of multiple genes.
- Not only recurrent molecular abnormities such as **FLT3, NPM1, CEBPA, RUNX1, and WT1**, numerous important new mutations have been also identified such as **DNMT3A, IDH1/IDH2, ASXL1, TP53, PTEN, HRAS, KRAS, and TET2** etc.
- often correlated with each other and have shown prognostic importance in AML.

- Among these genetic abnormalities, the **IDH1 mutation** was first revealed in more than 70% of **GBM patients**, providing **favorable prognostic predictions** as a stable and positive indicator than those with wild-type IDH1 genes.

- The **prevalence and prognostic value of IDH1 mutation in adult AML** has remained contradictive despite numerous reports since the acquired genetic mutations in IDH1 have been subsequently identified in AML.

- It has been reported that mutations of IDH1 and IDH2 in AML occur in approximately 15 to 30 % in adult patients and substantially lower in pediatric patients (< 2%).

- **MicroRNA (miRNA)** is about 19 to 25 long noncoding RNAs that post-transcriptionally regulate the expression of target genes

- Recently, **IDH1 mutation-specific miRNA signatures** have been revealed as a **favorable prognostic marker** in **GBM patients** with wild-type IDH1, thus predicting a subgroup of IDH1 wild-type GBM patients that showed better survival outcomes similar to patients with IDH1 mutations.

- Several studies have shown the importance of **miRNA in AML** and identified several miRNAs as new potent biomarkers associated with efficient diagnosis and clinical outcome from the prognostic standpoint.

- IDH1 mutation-specific miRNA signatures have not been reported nor evaluated for the prognostic values of AML patients with wild-type IDH1.

- a cohort of 200 clinically annotated **de novo adult AML cases** were available through The Cancer Genome Atlas **(TCGA) data portal** (https://tcga-data.nci.nih.gov/tcga/).

- We first investigated the distribution of patients with **IDH1 mutations** in relationship with **cytogenetic risk categories** as well as **in molecular subgroups**.

- We also examined the **prognostic impact of IDH1 mutations** on survival.

- Using significance analysis of microarray (SAM) method, we have further investigated **IDH1 mutation-specific miRNA signatures** as complimentary potent biomarkers and validated the predictive impact of these signatures on the survival outcomes of AML patients with wild-type IDH1.

MAERIALS AND METHODS

TCGA miRNA dataset
and clinical sample information

- **Whole-genome microRNA expression profiles** and **the corresponding clinical data** for acute myeloid leukemia (AML) cases were downloaded from The Cancer Genome Atlas (TCGA) data portal site (https://tcga-data.nci.nih.gov/tcga/) in January, 2014.
- The total number of patients provided in the TCGA was 200
- the available miRNA data samples were 188.

- total **184 patients** who had both clinical information and corresponding miRNA-seq data were considered for further analysis throughout the study.

Comparisons of overall survival analysis

- we grouped samples
  - by three cytogenetic risk categories
    - favorable risk, normal/intermediate risk, and poor risk group
  - by different molecular genetic abnormalities
    - FLT3 (135 negative vs. 58 positive samples), NPM (150 negatives vs. 46 positives), IDH1 R132 (177 negatives vs. 18 positives), IDH1 R140 (181 negatives vs. 15 positives), IDH1 R172 (193 negatives vs. 3 positives), Activating Ras (186 negatives vs. 11 positives), BCR-ABL (15 negatives vs. 1 positive sample), and PML-RAR (9 negatives vs. 8 positives)
  - We considered a patient as one who had IDH1 mutation if he or she had one of the three types of mutations.

Analysis of miRNA expression profiles

- **Out of 705 miRNAs** available for patient samples, genes with zero read count values were removed and **176 miRNAs** were remained and used for further analysis.

- This raw miRNA read count data was quantile-normalized and used as input for plotting abundance heatmaps of their differential expressions using 'clustergram' function in MATLAB software.

- **Differential expression profiling analysis of miRNAs** performed on **cytogenetically normal AML** patient group (n = 107; 21 mutant IDH1 and 86 wild) using significance analysis of microarrays (SAM).

- Two-class unpaired SAM design applied in this study is <u>**to pick out miRNAs whose mean expression level is significantly different between mutant-type IDH1 patient group and wild-type IDH1**</u> patient group.

survival prediction of IDH1 mutation-specific miRNA signature

- To assess **prognostic effect of selected IDH1 mutation-specific miRNAs**, we assigned each patient a **risk-score** that is a linear combination of the expression levels of the significant miRNAs weighted by the SAM d-values.

- Cytogenetically poor-risk AML patients **(CP-AML) without IDH1 mutation** were used as a testing group.

- Risk scores of 32 CP-AML wild-type patients were ranked from low to high. Then the CP-AML wild-type IDH1 patients were divided into two groups, low-score group and high-score group and analyzed their survival days.

RESULT

Incidence of mutations in AML patients

Mutations	Total no	Negative	Positive
FLT3	193	135	58 (30%*)
NPM1	196	150	46 (23.5%)
IDH1 R132	195	177	**18 (9.2%)**
IDH1 R140	196	181	**15 (7.7%)**
IDH1 R172	196	193	**3 (1.5%)**
BCR-ABL	16	15	1
Activating RAS	197	186	11 (5.6%)
PML-RAR	17	9	8

About 17% of AML patients in TCGA had IDH1 mutations

IDH1 mutations in association with cytogenetic risk categories and other genetic alterations

|	Cytogenetic-risk Categories					
	favorable-risk (n=35)		intermediate- / normal-risk (n=107)		poor-risk (n=42)	
		IDH1 +		IDH1 +		IDH1 +
FLT3-negative & NPM-negative	24 (684[#])	0	46 (319)	9 (365)	34 (212)	7 (n/a)
FLT3-negative & NPM-positive	0	0	20 (410)	6 (731)	1 (n/a)	0
FLT3-positive & NPM-negative	11 (30)	0	17 (135)	0	5 (n/a)	2 (n/a)
FLT3-positive & NPM-positive	0	0	19 (243)	5 (n/a)	2 (n/a)	1 (n/a)

Frequencies of IDH1 mutations

- We found that **about 17% of AML** patients in TCGA had IDH1 mutations. Of these, there was **no occurrence of IDH1 mutations in cytogenetically favorable-risk group** of patients, but in cytogenetically normal-risk group (19%) and in cytogenetically poor-risk group of samples (23%).
- In adult AML, it has been previously reported that the occurrence of IDH1/2 mutations has been observed only occasionally (<1%) in favorable risk patients.
- **Neither NPM1 mutations** were observed **in favorable risk group**.
- Our results on the dual absence of NPM1 and IDH1 mutations in favorable risk group are in line with many previous studies on adult AML patients that have already described about a strong co-occurrence between NPM1 and IDH1/2 mutations.
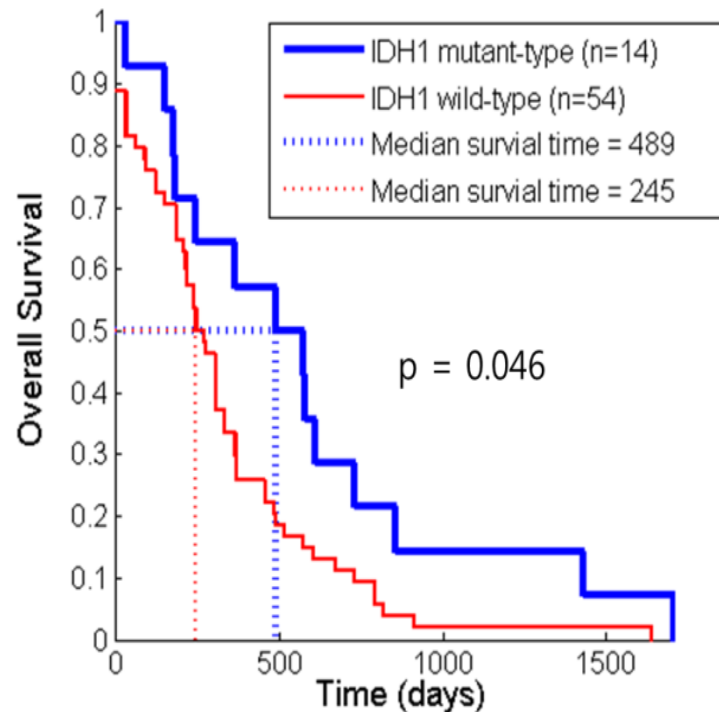
Prognostic value of cytogenetic profiles in TCGA AML patients
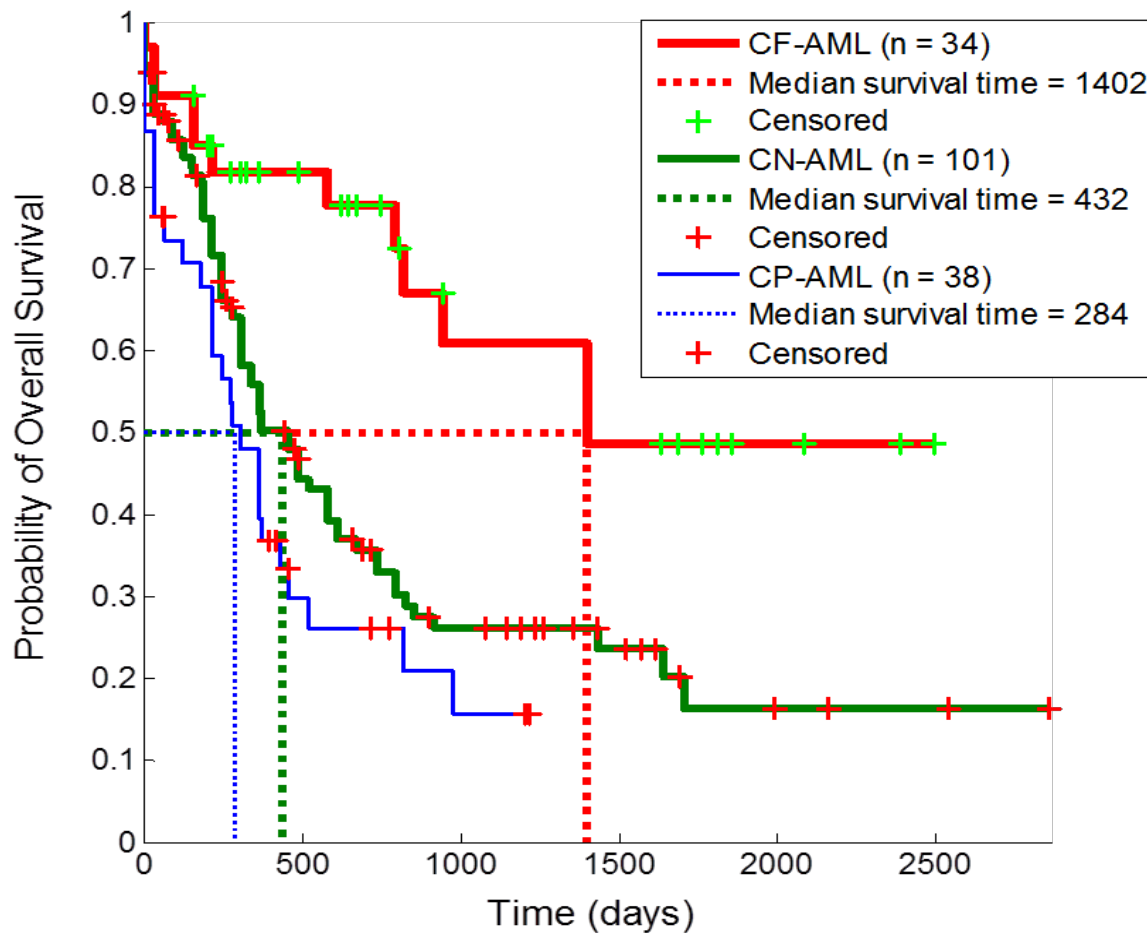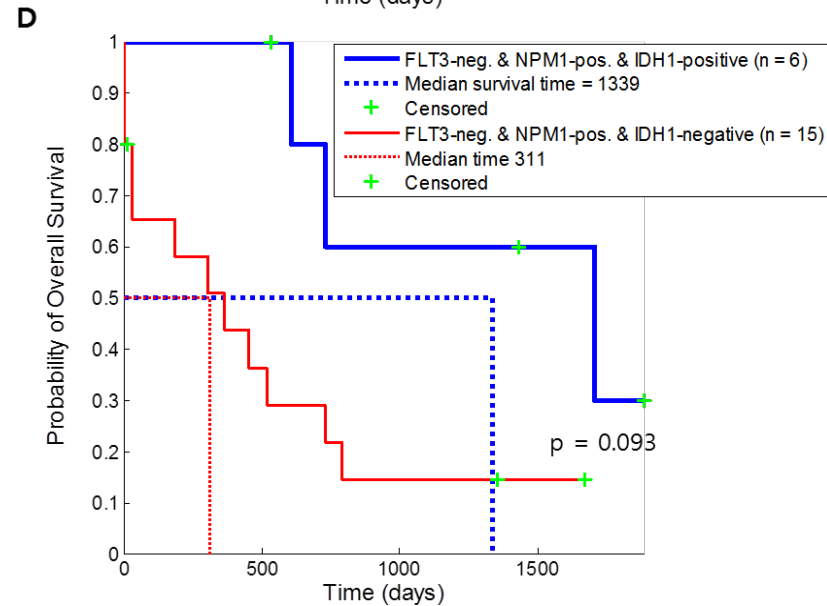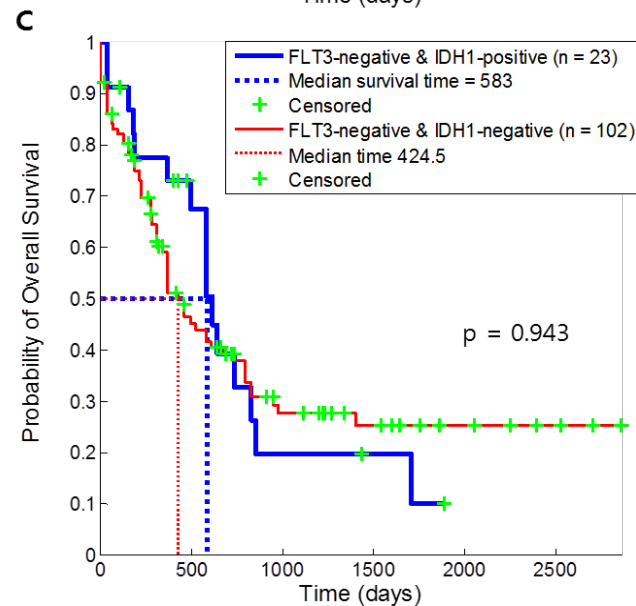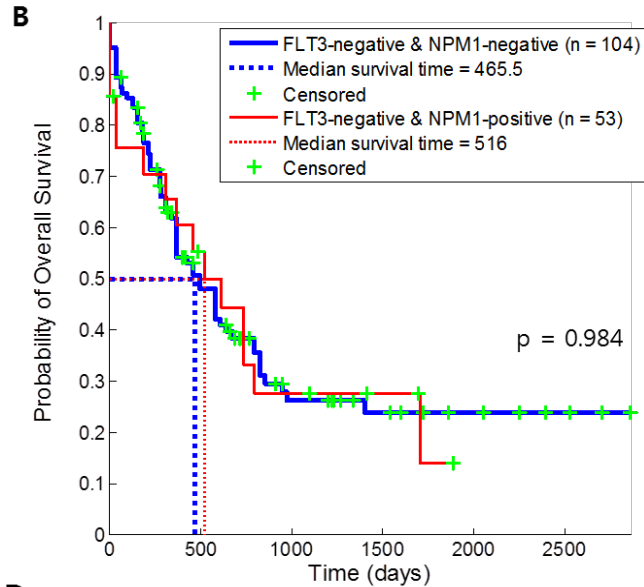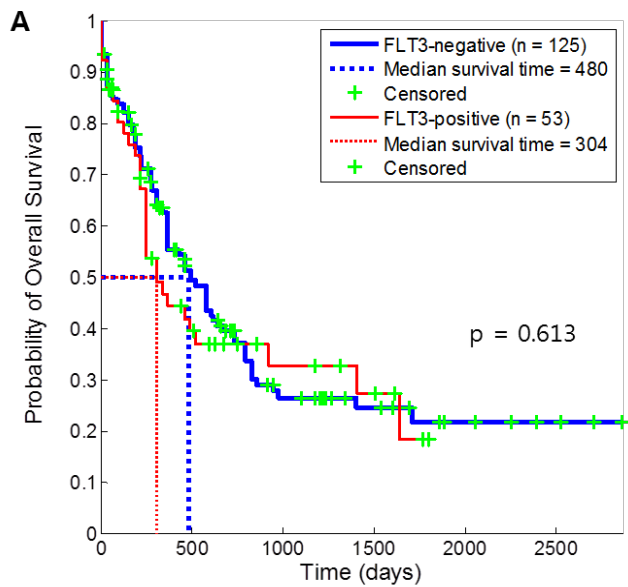
- IDH1 mutations in **combination with the known molecularly low-risk genetics (i.e., FLT3-negative and NPM1-positive)** predicted dramatically improved favorable prognostic outcomes.
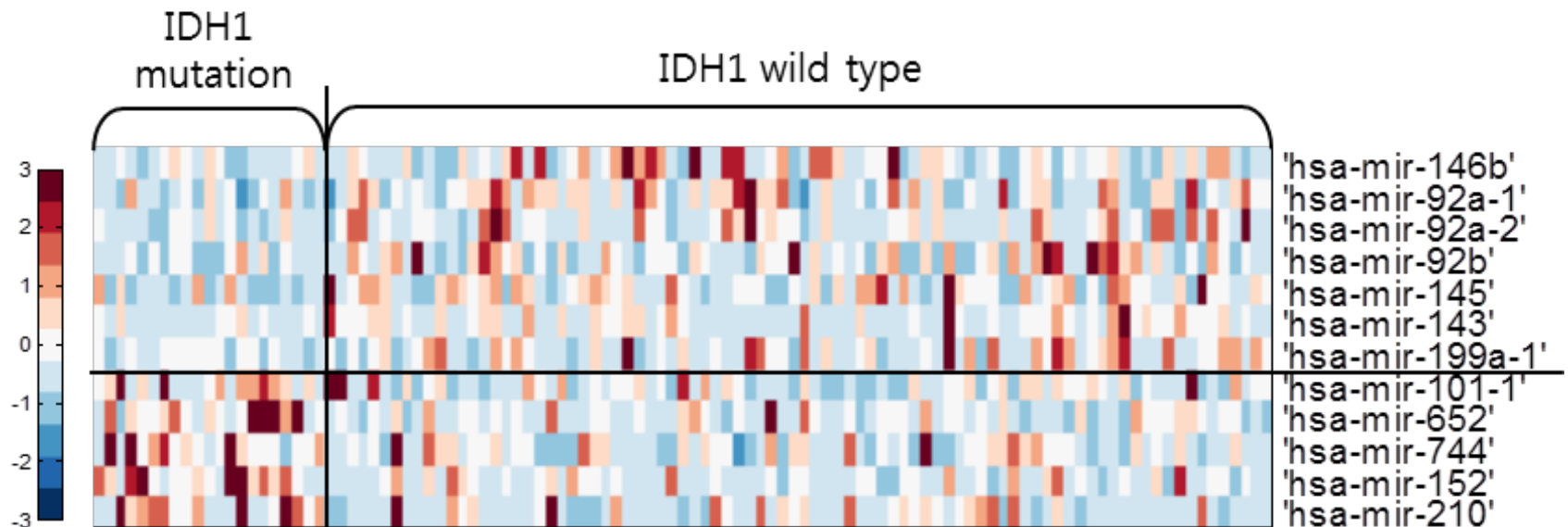
Kaplan-Meier estimates for overall survival of CN-AML patients in the IDH1 mutant-type samples and wild-type patients


- **IDH1 mutant-type group showed better survival** than the wild-type group.

# IDH1 mutation-specific twelve miRNA signature from CN-AML patients

- Five miRNAs of **miR-101-1, miR-152, miR-210, miR-652, and miR-744** were protective, expressing higher in patients with IDH1 mutation (low-risk group)

- seven miRNAs of **miR-143, miR-145, miR-146b, miR-199a-1, miR-92a-1, miR92a-2, and miR92b** were risky, expressing higher in patients with wild-type IDH1 (high-risk group)

Evaluation of the value of 12 miRNA expression signature for survival prediction using cytogenetically poor-risk group of AML patients with wild-type IDH1
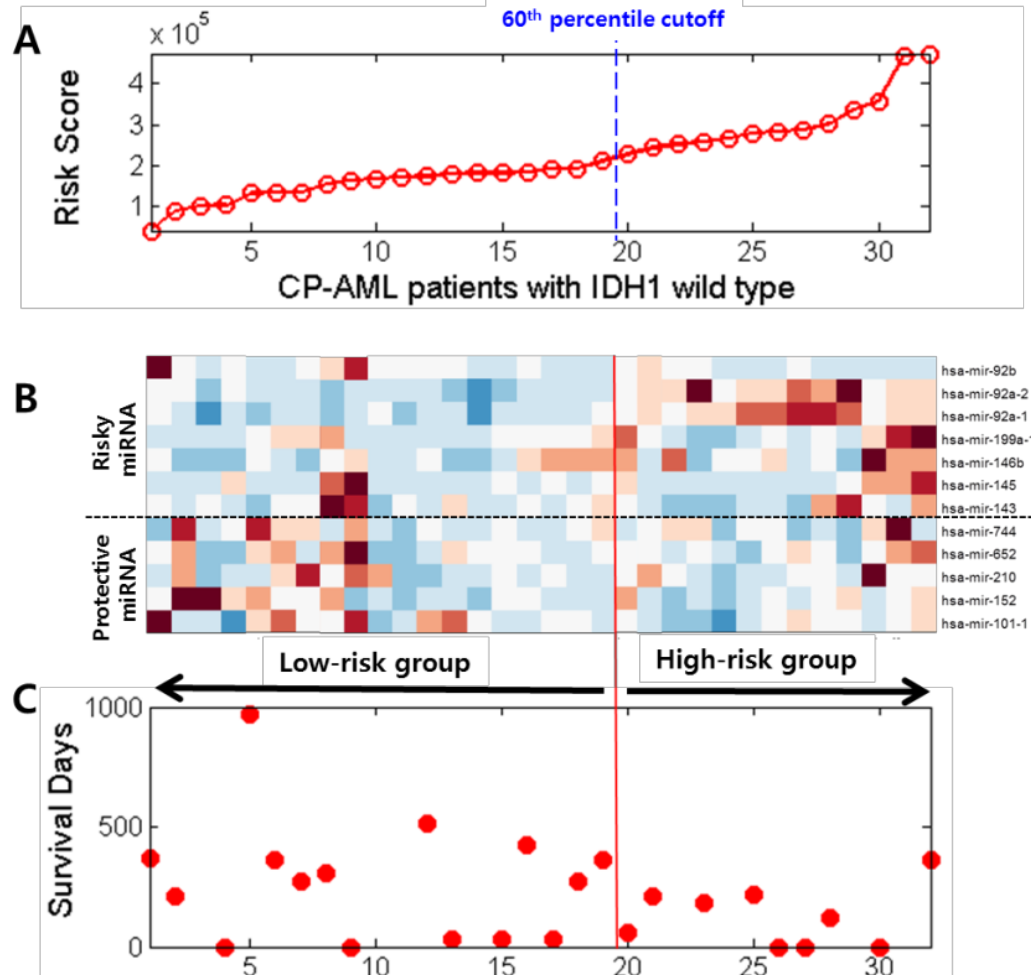
Kaplan-Meier estimates between low-risk and high-risk groups for overall survival of CP-AML patients with IDH1 wild type
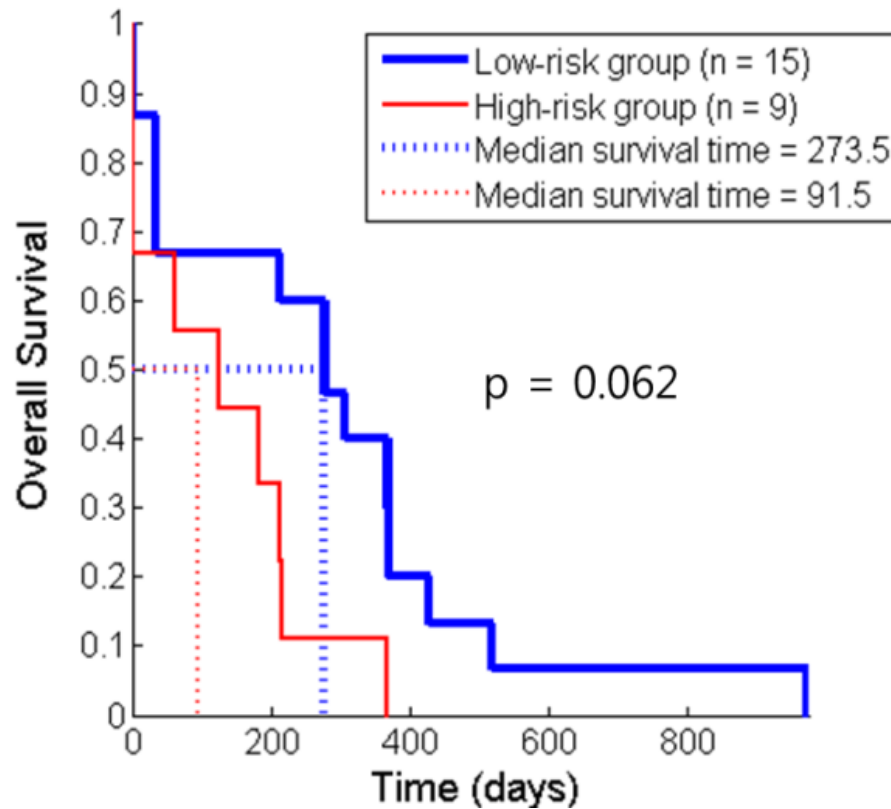
Essential miRNA analysis

- To examine whether all miRNAs are required in the signature, we removed one miRNA at a time from the twelve miRNA set using the testing data (CP-AML group) and constructed 12 testing set of 11 miRNA signature.

- We then ranked these testing samples according to the calculated risk-scores with 11 miRNA signature after leaving-one-out.

- The results showed that 10 set of 11 miRNA signature showed the same marginal significance for overall survival like the 12-miRNA signature analysis (p = 0.062).

- However, when miR92a-2 was removed, the survival outcome between low-risk and high-risk group was not statistically significant (p = 0.920), indicating that the **miR92a-2** as an essential miRNA was highly correlated with the favorable survivals.

Potential role of the five protective miRNAs
(miR-101-1, miR-152, miR-210, miR-652, and miR-744)

- **miR-101-1** has been previously reported to regulate p23, which was in a high level in leukemic cells. Liu et al has reported that p23 was post-transcriptionally down-regulated by miR-101 and subsequently associated with reduced in pediatric ALL cases. Thus, the up-regulation of this miR-101 may provide a favorable prognosis of patients.

- **miR-152** has been reported as a tumor suppressor that targeted DNA methytransferase DNMT1 and the restoration of miR-152 expression has shown to be sufficient to inhibit tumor cell growth in solid tumor.

- **miR-210** expression was significantly lower in patients suffering from relapse and induction failure than in other patients among childhood acute lymphoblastic leukemia (ALL).

- The down-regulation of **miR-652** was observed in patients with chronic liver disease, and selective expression of miR-652 was observed in innate immune cells. Its impact on hematological malignancies has not been reported yet.

- Overexpressing **miR-744** was identified to significantly inhibit endogenous TGF-β1 synthesis in various human tissues. Thus, the role of this miR-744 might be involved in inhibiting TGF-β1-directed cellular responses and tumorigenesis.

seven miRNAs of <u>miR-143, miR-145, miR-146b, miR-199a-1, miR-92a-1, miR92a-2, and miR92b</u> were risky

- **<u>miR-199a</u>** was found to be <u>associated with worse overall survival in AML patients with predominantly intermediate- and poor-risk cytogenetics</u> than those with low expression.

- While expression of **<u>miR-143/-145</u>** cluster has been greatly <u>reduced in several cancers including AML</u> patient samples and shown to <u>possess antitumorigenic activity</u>, other reports have indicated the high levels of expression of mature **miR-143** and **miR-145** were associated with recurrence of metastasis in esophageal squamous cell carcinoma (ESCC) patients and the up-regulation of **miR-143** transcribed by NF-kB promoted cancer migration an tumor metastasis in hepatitis B virus-relate hepatocellular carcinoma (HBV-HCC).

- **miR146b** was highly expressed in adult papillary thyroid carcinomas and correlated to poor prognosis in squamous cell lung cancer and glioblastoma.
- **miR-92a-1** has been reported as a key oncogenic component in various cancers, including *non-Hodgkin lymphomas* and colon cancer. Higher level of miR-92a-1 in patients with colorectal cancer and advanced adenoma was independently associated with poor survival.
- Higher tumor **miR-92a-2** levels are associated with chemoresistance and with decreased survival in patients with small cell lung cancer (SCLC). **miR-92b** has been considered as a potential tumor oncogene to promote GBM cell proliferation and was significantly up-regulated in non-small cell lung cancer (NSCLC) playing an oncogene roles for cell growth, cisplatin chemosensitivity phenotype.

CONCLUSION

- Taken together, **IDH1 mutations were only identified in CN-/CP-AML** groups in TCGA

- IDH1 mutations have predicted **favorable outcomes** when combined with molecularly low-risk (FLT3-negative and NPM1-positive) group.

- Using selected **twelve IDH1 mutation-specific miRNAs signatures** obtained from CN-AML, we have tested CP-AML wild-type patients regarding their survival outcomes and **successfully identified a subgroup of patients with better outcomes** among the poor-risk group.

- Our findings may add prognostic or therapeutic implications for the future evaluation of AML patients.

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