Acute Myeloid Leukemia

The prognostic value of cN-II and cN-III enzymes in adult acute myeloid leukemia

We analyzed the expression of deoxycytidine kinase (dCK), UMP/CMP-kinase (UMP/CMP-K), nucleotide diphosphokinase (NDPK-B) and 5'-nucleotidases cN-II, cN-III, cdN and mdN by quantitative polymerase chain reaction at diagnosis in leukemic blasts from 96 patients with acute myeloid leukemia (AML) treated with ara-C. Our results show that high mRNA levels of cN-II and low mRNA levels of cN-III are correlated with a worse clinical outcome and suggest that these enzymes may have a role in sensitivity to ara-C in AML patients.

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Substrate cycle enzymes participate in the activation of many nucleoside analogs used in the treatment of hematologic malignancies, including ara-C.[1] Alterations in the expression of these enzymes may affect the accumulation of the active triphosphate form, ara-CTP, and thus, modifiy the therapeutic effectiveness of ara-C.[2] We assessed the level of mRNA expression of three kinases (dCK, UMP/CMP-K, NDPK-B) and four 5'-nucleotidases (cN-II, cN-III, cdN and mdN) involved in substrate cycles by quantitative real time reverse transcriptase polymerase chain reaction (RT-PCR) in leukemic blasts obtained at diagnosis from bone marrow aspirates (n=70) and peripheral blood (n=26) of 96 AML patients treated with various ara-C-containing regimens. The separation of bone marrow blasts, extraction of cellular RNA, cDNA synthesis and real-time PCR analysis were performed as previously described[3] using the primers and probes listed in Table 1. In 20 patients, *in vitro* sensitivity of fresh leukemic blasts cells to ara-C cytotoxicity was measured as described previously using the 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl-tetrazolium bromide (MTT) assay.[4]

The patients' characteristics are listed in Table 2. In univariate analysis, high levels of cN-II mRNA at diagnosis were correlated with a worse disease-free survival (hazard ratio: 1.5; CI95: 1.1-2.08; p=0.007). High levels of cN-II mRNA were also correlated with a worse overall survival (hazard ratio: 1.2; CI95: 1.02-1.5; p=0.02), while high levels of cN-III mRNA were correlated with a better OS (hazard ratio: 0.5; CI95: 0.3-0.9; p=0.03). In multivariate analysis, high cN-II mRNA levels were associated with a worse overall survival (hazard ratio: 2; CI95: 1.3-3; p<0.01) whereas high levels of cN-III mRNA were associated with a better overall survival (hazard ratio: 0.5; CI95: 0.2-0.9; p=0.04). *In vitro* cytotoxicity assays demonstrated a significant correlation only between high cN-II

Table 1. Primer sequences and PCR profiles for different factors implicated in gemcitabine drug resistance.

Gene	Primer sequence	Probe
dCK	for: aaa cct gaa cga tgg tct ttt tac c rev: ctt tga gct tgc cat tca gag a	5'-Fam-caa aca tat gcc tgt ctc agt cga ata aga gct c-Tamra
UMP/CMP-K	for: gggcatattctttgcttcca rev: tgcatttcaaggttccactg	SYBR Green
NDPK-B	for: atgcagtgcggcctggtggg rev: gacccagtcatgagcacaagac.	SYBR Green
cN-II	for: acc tgc tgt att acc ctt tca gct a rev: gct cca ccg ttg att catg a	5'-Fam-ctc ttc agg gct gcc cat gtc ttg a-Tamra
cN-III	for: aatcggcgatgtactagag rev: catctgccattcttaagtctc	SYBR Green
cdN	for: gga cacgcaggt ctt cat ctg rev: gcg gta ctt ctc acc cac aca	5'-Fam-cca gcc ccc tgc tga agt acc acc-Tamra
mdN	for: catcagcatttgggagtcaa rev: cgacacaatctgctccagaa	5'-fam-cgtcttcatctgcacaagcccca-tamra

Table 2. Laboratory characteristics of acute myeloid leukemia patients at diagnosis.

	Median
Age, mean ± SD (years)	55.1±17.6
Complete response rate to induction therapy	70%
Disease-free survival (months)	11.3
Overall survival (months)	11.5
White blood cell count ($\times 10^9$/L)	37.8
Peripheral blood blasts (%)	60
Bone marrow blasts (%)	80
Hemoglobin (g/L)	86
Platelets ($\times 10^9$/L)	60
Cytogenetic risk (n=81)	N (%)
Low	13 (16)
Intermediate	38 (47)
High	30 (37)

mRNA expression and high IC_{50} values for ara-C (r= 0.59; p=0.007).

The results presented here in a new series of patients confirm our previous data reporting that high expression of cN-II mRNA was corrrelated with a worse prognosis in adult AML patients.[3,5-7] It is not clear at present whether the prognostic significance of cN-II is due to the fact that this enzyme may dephosphorylate ara-CMP or whether it reflects the proliferation status of the AML clone, given that cN-II is involved in substrate dNTP cycles and may act as a marker of disease aggressiveness. Our finding demonstrating a significant correlation between high cN-II mRNA expression in leukemic blasts and high levels of ara-C IC_{50} in *in vitro* cytotoxicity tests may suggest a role of this enzyme in the resistance to ara-C in AML patients. However, we did not find a direct correlation between cN-II expression and response to induction treatment. Moreover, a recent study by Mazzon reported that *in vitro* cN-II does not dephosphorylate ara-CMP, suggesting that the role of cN-II in resistance to ara-C is not due to direct degradation of ara-CMP.[8] A second possibility is that the survival of highly-proliferating cN-II positive cells after induction treatment may favor rapid regrowth of disease, shortening the disease-free survival and, subsequently, overall survival.

The results demonstrating that low levels of cN-III were related to a worse overall survival were unexpected. A possible explanation of our results is that accumulation of pyrimidine nucleotides associated with low cN-III levels may cause differential sensitivity to ara-C. In fact, low levels of cN-III would lead to higher levels of dCTP, favoring the survival of blast cells exposed to ara-C and consequently higher resistance to induction treatment.[9] Another possible explanation could be the phosphotransferase activity of cN-III that phosphorylates ara-C to ara-CMP.[10] According to this hypothesis, low levels of the enzyme would also imply lower ara-C activation and activity. A final explanation could be that in fact cN-III is not involved in ara-C metabolism as we did not show a relationship between enzyme mRNA expression and response to induction treatment or disease-free survival. In fact, cN-III mRNA levels were correlated only with overall survival indicating that this enzyme may only reflect disease aggressiveness.

In summary, our results demonstrate a relationship between cN-II and cN-III expression and the prognosis of adult patients with AML. Whether this is due to a modification in the activation of ara-C or a modification of the dNTP pools in blast cells is not clear. It is expected that future work will reveal the specific role of cN-II and cN-III in the prognosis of adult patients with AML.

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