The prevalence and clinical relevance of p27 and cyclin E immunoreactivity in myelodysplastic syndromes

We report on p27 and cyclin E immunoreactivity (IR) in 86 patients with myelodysplastic syndromes (MDS). No association was found between cyclin E IR and survival in the whole series. Cyclin E IR was more prevalent in patients with refractory anemia (RA) and RA with ringed sideroblasts (RARS) with poor survival, although this trend was not statistically significant.

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Genetic alterations of the cell cycle regulators p27 and cyclin E have never been reported in hematopoietic malignancies, but their abnormal expression is associated with a poor prognosis in non-Hodgkin's lymphomas[1] and acute myeoid leukemia (AML).[2] We immunohistochemically analyzed p27 and cyclin E immunoreactivity in nitric acid-decalcified, Bouin-fixed and paraffin-embedded bone marrow biopsies of 86 MDS patients (23 with RA, 10 with RARS, 39 with RA with excess blasts (RAEB), 6 with RAEB in transformation (RAEB-t), and 8 with chronic myelomonocytic leukemia (CMML). Seventy-eight consecutive patients were retrieved from the files of the Ospedale Maggiore, the only inclusion criterion being the availability of a bone marrow biopsy at diagnosis. The remaining eight patients were retrieved from the 1999 files of S. Raffaele Hospital Scientific Institute, and selected according to the availability of a bone marrow biopsy at diagnosis and subsequent development of acute myeloid leukemia. Overall, there were 45 males and 41 females with a mean age of 66 years (range 18-86). The follow-up was available for 76 (88.3%) patients and its mean time was 56.7 months (range 1.1-149.0). Thirty-nine patients (45.3%) progressed to AML in a mean time of 29 months (1-79). Thirty-seven (48.6%) patients died because of their disease and 39 were alive with disease. IR for p27 and cyclin E was assayed by means of the ABC method with the anti-p27 57 (Transduction Laboratories) and anti-cyclin E 13A3 (Novocastra Laboratories) monoclonal antibodies (working dilution: 1:200 and 1:50, respectively). At least 500 cells were evaluated at x1000 magnification, and only nuclear staining was considered positive. Control experiments were performed on normal bone marrow biopsies from ten Italian patients (three men and seven women, mean age 52 years) with localized solid tumors (six with infiltrating duct carcinoma of the breast and four with small cell carcinoma of the lung). For statistical purposes, the threshold for the variables of hemoglobin, M/E ratio and cyclin E IR was established according to their median values in MDS (Table 1). The associations between the clinico-pathologic and immunohistochemical data were evaluated by the Mann-Whitney test, χ^2 test or Fisher's exact test. Survival estimates were calculated with Kaplan-Meier's method and compared by the log-rank test. The Cox proportional hazard regression model was used to evaluate the simultaneous effect of explanatory variables on survival time.

In normal bone marrow, IR for p27 was detected in endothelial cells, lymphocytes, plasma cells and in the vast majority (mean 85%, standard deviation ±8%) of megakaryocytes, whereas erythroid and myeloid cells were invariably unreactive, a finding in keeping with previous studies.[3] In MDS, erythroid and myeloid cells were also unreactive and p27 IR was detectable in most of the dysplastic megakaryocytes, although to a lesser extent than in normal controls (78±16%, p=0.177).

In normal bone marrow, cyclin E IR was detectable in a very small fraction (4±1%) of intermediate and immature cells of the erythroid and myeloid lineages,[4,5] whereas megakaryocytes were always unreactive. In MDS, the percentage of immunoreactive cells was significantly higher (10±7% vs. 4±1%, p=0.008). In particular, the values of cyclin E IR were 9±7% in RA, 13±10% in RARS, 11±7% in RAEB, 7±7% in RAEB-t and 6±3% in CMML. The RA (p=0.014), RARS (p=0.011) and RAEB (p=0.003), but not RAEB-t (p=0.194) and CMML (p=0.064) subtypes had values of cyclin E IR significantly higher than those in normal controls. There was a significant difference in cyclin E IR between CMML and the other MDS subtypes (6±3 vs. 11±7, p=0.028), and not among the subtypes of RA, RARS, RAEB and RAEB-t (p=0.418), a finding in line with the notion that CMML represents a disorder distinct from MDS.[6] Cyclin E has been recently identified as a target for activation by ionizing radiation, playing a functional role in apoptosis of hematopoietic cells.[7] This finding leads to speculation that cyclin E up-regulation may contribute to ineffective hematopoiesis in MDS by trigging apoptosis, a pathway that is activated in these disorders, particularly in RA/RARS subtypes.[8] At univariate analysis, no significant association was found between cyclin E IR, risk of progression (p=1.000) or time to progression (p=0.587) towards AML.

No significant correlation was found between cyclin E IR and the clinico-pathologic variables analyzed. At univariate

Table 1. Univariate analysis (Kaplan-Meier) in the whole series.

	Patients	*Deaths*	*Log-rank*
Gender			
Males	40	24	
Females	36	13	0.0257
Age			
<60	25	10	
≥60	51	27	0.3236
IPSS[1]			
Low/INT-1	45	13	
INT-2/High	23	20	<0.0001
Myeloblasts			
<5%	28	4	
5-10%	28	16	
>10%	20	17	<0.0001
Hb[2]			
<10.7	35	19	
≥10.7	37	17	0.5317
M/E[3] ratio			
<1.35	34	11	
≥1.35	36	24	0.0011
Cyclin E IR[4]			
<9%	37	18	
≥9%	39	19	0.8751
FAB subtype			
RA	18	2	
RARS	10	4	
RAEB	36	21	
RAEB-t	4	4	
CMML	8	6	<0.0001
Karyotype			
46, XX/XY	36	14	
Others	40	23	0.1197

[1]International Prognostic Scoring System;[2]Hemoglobin g/dL;
[3]Myeloid/erythroid ratio;[4]Immunoreactivity.

analysis, the International Prognostic Scoring System (IPSS),[9] percentage of blasts, M/E ratio, FAB subtype, but not cyclin E IR, were significantly associated with survival (Table 1). Multivariate analysis showed that IPSS (p=0.0068) and RAEB-t subtype (p=0.001) were independent predictors of survival. Interestingly, when the survival analysis was restricted to the low-risk subset of RA/RARS, the variables of age (p=0.0287), IPSS (p=0.0113) and FAB subtype (RA vs. RARS, p=0.0563) were associated with survival. At multivariate analysis, the variable of age (p=0.0284) was the only independent predictor of survival in RA/RARS. Although cyclin E IR was more prevalent in RA/RARS with an adverse clinical course, this trend did not achieve statistical significance, either at univariate (p=0.0759), or multivariate (p=0.0604) analysis, possibly because of the relatively small number of cases.

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Incidence of trisomy 8 and 9, deletion of D13S319 and D20S108 loci and BCR/ABL translocation in non-treated essential thrombocythemia patients: an analysis of bone marrow cells using interphase fluorescence *in situ* hybridization

We compare conventional cytogenetics (CC) with fluorescence *in situ* hybridization (FISH) in 53 untreated patients with essential thrombocythemia. CC revealed no abnormalities. When FISH was used, no BCR/ABL rearrangement nor trisomy 8 was found, but one trisomy 9, two del(13)(q14) and five del(20)(q12) were observed. FISH detected chromosome abnormalities in 15% of patients in which no alteration was found by CC.

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Essential thrombocythemia (ET) is a chronic myeloproliferative disorder (CMPD) with megakaryocytic proliferation in bone marrow resulting in a persistent increase in platelets in peripheral blood. In ET patients chromosome abnormalities detected by G-banding are rare, and no specific abnormality has been identified.[1] Only about 5% of patients show an abnormal karyotype at diagnosis.[2] The most frequent cytogenetic anomalies detected by conventional cytogenetics (CC) are trisomies of chromosomes 8 and 9 and deletions in 13q and 20q.[1]

The finding of an abnormal karyotype would be useful to distinguish ET from secondary thrombocytosis as it gives a clonal hallmark to the disease. For this reason, we re-evaluated genetic findings obtained by CC using fluorescence *in situ* hybridization (FISH) probes, with the aim of yielding more information about chromosomal abnormalities in ET patients.

Herein, we present 53 cases diagnosed as having ET according to the *Polycythemia Vera Study Group* (PVSG) criteria[3] and who had not previously received cytolytic treatment. Samples from ten healthy volunteers were used as assay validation controls. Chromosome analyses were carried out on hematologic cells from 24-hour bone marrow cultures. Karyotypes were described according the International System for Human Cytogenetic Nomenclature.[4]

FISH studies were performed on fixed nuclei from CC following the standard procedures (Table 1). CC results were available in 49/53 patients, all showing a normal karyotype; in the remaining four cases no metaphases were obtained. When FISH was performed, no patient showed BCR/ABL rearrangement nor trisomy 8. One patient showed trisomy 9 in 30% of studied cells, in 2/53 patients a 13q14 deletion was found (frequencies 16% to 26.5%) and 5/53 patients presented a 20q12 deletion (frequencies 10.5% to 13.5%). Both monosomies were hemizygous. There were no cases with more than one abnormality. FISH probes detected chromosomal abnormalities in 8/53 ET patients (15.1%) (Table 2).

FISH studies have been done in ET patients in order to search for chromosome 8 and 9 abnormalities.[5,6] Elis *et al*.[5] reported