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SHORT REPORT

Aberrant *p15* gene promoter methylation in therapy-related myelodysplastic syndrome and acute myeloid leukaemia: clinicopathological and karyotypic associations

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Summary. Seventeen patients with therapy-related myelodysplastic syndrome/acute myeloid leukaemia (t-MDS/AML) were examined for aberrant *p15* gene methylation by methylation-specific polymerase chain reaction. Ten patients (58%) showed *p15* methylation, which was significantly related to monosomy/deletion of chromosome 7q, but not to antecedent chemotherapy, blast count, leukaemic evolution or survival. In three of six patients with marrow samples obtained prior to the diagnosis of t-MDS/AML, *p15* methylation predated disease development by up to 2 years. Bone marrow transplantation led to the disappearance of *p15* methylation in one patient. These results showed that *p15* methylation was an early event in the evolution of some t-MDS/AML patients.

Keywords: therapy-related acute myeloid leukaemia/myelodysplasia, *p15* methylation.

Therapy-related myelodysplasia and acute myeloid leukaemia (t-MDS/AML) are distinct disorders in the World Health Organization (WHO) classification of myeloid malignancies. Alkylating agent-related t-MDS/AML has a longer latency, and is associated with monosomies/deletions of chromosomes 5 and 7. Topoisomerase II inhibitor-related t-MDS/AML has a shorter latency and is associated with *MLL* gene rearrangements. Both types are characterized by multilineage dysplasia, a preleukaemic phase and a poor prognosis. In addition, t-AML cases with well-defined cytogenetic abnormalities, including t(15;17)(q22;q21), t(8;21)(q22;q22) and inv(16)(p13q22), also occur (Rowley & Olney, 2002). They are, however, comparable clinicopathologically and prognostically to *de novo* AML with similar cytogenetic abnormalities.

Aberrant promoter CpG methylation, leading to transcriptional silencing, is increasingly recognized as an important pathogenetic event in AML. The *p15* gene, critically involved in cell cycle regulation, is commonly methylated in *de novo* AML, occurring in up to 93% of patients (Chim *et al*, 2001).

In this study, we investigated a consecutive series of t-MDS/AML to define the frequency and time course of aberrant *p15* methylation in this disorder.

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MATERIALS AND METHODS

Patients. t-MDS/AML were diagnosed according to WHO classification criteria. Cytogenetic analyses were performed on overnight unstimulated cultures of marrow cells. Metaphases were Giemsa banded and karyotyped according to the International System for Human Cytogenetic Nomenclature.

Methylation-specific polymerase chain reaction (MSP) for aberrant p15 methylation. MSP for aberrant p15 methylation was performed on marrow DNA as described (Chim *et al*, 2001). Briefly, 1 μg of genomic DNA was modified with bisulphite (CpGenome DNA modification kit; Intergen, USA), purified and subjected to MSP that detected the methylated (M) and unmethylated (U) promoter sequences. Positive and negative controls included universally methylated DNA (Intergen) and normal donor DNA respectively. The sensitivity of the method was estimated to be 10^{-3}–10^{-4} (Chim *et al*, 2001).

RESULTS

Patients, clinicopathological features and treatment outcome

Seventeen consecutive patients were studied (Table I), five of whom have been reported briefly (Kwong *et al*, 1998; Au *et al*, 2000, 2001a,b). Antecedent chemotherapy included single agents (azathioprine/cyclophosphamide), combination chemotherapy and conditioning regimens for

Table I. Clinicopathological and karyotypic features of 17 patients with t-MDS/AML.

Patient number/ sex/age (years)	Primary disease	Drugs	Latency	t-MDS/ AML	Blasts*	Karyotypic aberrations†	*p15* methylation/Time from t-MDS/AML	Treatment	Outcome	Survival‡
1 M/42	Crohn's disease	Aza, CTX, Sulphasalazine	7 years	RCMD	2%	–Y, +8, +9	+/0 m (+)	Nil	NR, alive	22 m+
2 M/71	NHL-MCL	mBACOD, RT, FND	4 years	RAEB-1	6%	add(3)(q27), add (4)(q21), −5, t(13; 22)(p11;q11), add(16)(p11),+mar	–/−26 m (–), −18 m (–), 0 m (–)	Nil	Died	2 m
3 M/43	NHL-DLBCL	CEOP, AutoBMT	9 years	RCMD	3%	−7	+/−59 m (–), −26 m (+), −19 m (+), 0 m (+), post-BMT (–)	BMT	CR	12 m
4 F/58	RA	Aza, CTX, MTX	13 years	RAEB-2	16%	−5, −7, i(10)(q10)	+/0 m (+)	Nil	Died	6 m
5 F/33	HL	COPP/ABVD	4 years	AML	20%	–X, −7, t(8;21)(q22;q22), inv(13)(q12q32), add(21)(q?), +r, +dmin	+/0 m (+)	BMT	Died	44 m
6 M/66	RA	Aza	8 years	CMML-2	12%	Nil	–/0 m (–)	Hydroxyurea	Died	1 m
7 F/59	SLE	Aza, CTX	6 years	RAEB-1	7%	+8, +21	+/−4 m (+), 0 m (+)	Hydroxyurea	Died	1 m
8 M/72	NHL-MCL	COPP	3 years	APL	90%	t(15;17)(q22;q21)	–/−15 m (–), 0 m (–), 6 m (–)	As_2O_3	CR	18 m+
9§ F/29	ALL	UKALL, AlloBMT	7 years	RAEB-2	15%	inv(3)(q21q26), del(5)(q13), add(17)(p11)	–/−48 m (–), −26 m (–), 0 m (–), 4 m (–), post-BMT (–)	BMT	CR	36 m+
10§ F/43	APL	Dauno, Ara-C, MTZ	7 years	AML	72%	del(5)(q13q33),7, add(9)(q31), add(17)(p?), del(21)(q21)	+/0 m (+)	Dauno, Ara-C	Died	7 m
11 M/65	NHL-FL	CVP, FND	6 years	APL	94%	t(15;17)(q22;q21)	–/0 m (–)	ATRA	Died	2 m
12§ F/44	SLE	Aza	10 years	AML	20%	−7, +21	+/0 m (+)	Dauno, Ara-C	Died	5 m
13 F/75	RA	CTX	11 years	RAEB-1	8%	t(5;12), −5, +i8q, +r	–/0 m (–)	Nil	Died	8 m
14 M/65	NHL-CLL	Chlorambucil, CVP	6 years	AML	46%	Normal	–/0 m (–),18 m (–)	Nil	Died	25 m
15 M/53	NHL-FL	CEOP, FND	4 years	RCMD	5%	del (7)(q22)	+/−37 m (–), −17 m (+), 0 m (+)	Nil	Lost to FU	14 m
16§ F/68	AMM	Melphalan, hydroxyurea	12 years	RAEB-2	6%	t(1;7)(q10;p10), − 7, + 8, + 12	+/0 m (+)	Nil	Died	8 m
17§ F/41	SLE	Aza	11 years	AML	60%	−7	+/0 m (+)	Dauno, Ara-C	Died	7 m

*Median blast count 15·4% for *p15* methylated *vs* 14·5% for *p15* unmethylated cases, P = not significant.

†−7/7q– in 8/10 *p15* methylated *vs* 0/7 *p15* unmethylated cases, p = 0·002 (Fisher's exact test).

‡Median survival 15 months in *p15* methylated *vs* 5·5 months in *p15* unmethylated cases, P = not significant.

§The cytogenetic results for these patients have been reported elsewhere. Details of these reports are available upon request from the authors.

M: male; F: female; NHL: non-Hodgkin's lymphoma; FL: follicular lymphoma; MCL: mantle cell lymphoma; DLBCL: diffuse large B-cell lymphoma; HL: Hodgkin's lymphoma; CLL: chronic lymphocytic leukaemia; RA: rheumatoid arthritis; SLE: systemic lupus erythematosus; ALL: acute lymphoblastic leukaemia; APL: acute promyleocytic leukaemia; AMM: agnogenic myeloid metaplasia; Aza: azathioprine; CTX: cyclophosphamide; m-BACOD: methotrexate, bleomycin, cyclophosphamide, vincristine, dexamethasone; RT: radiotherapy; FND: fludarabine, mitoxantrone, dexamethasone; CEOP: cyclophosphamide, epirubicin, vincristine, prednisolone; MTX: methotrexate; MTZ: mitoxantrone; AutoBMT: autologous bone marrow transplantation; AlloBMT: allogeneic BMT; COPP: cyclophosphamide, vincristine, prednisolone, procarbazine; Dauno: daunorubicin; ABVD; adriamycin, bleomycin, vinblastine, dacarbazine; UKALL: UK ALL regimen; Ara-C: cytosine arabinoside; CVP: cyclophosphamide, vincristine, prednisolone; yr: years; m: months.

bone marrow transplantation (BMT). Only one patient had received radiotherapy. The median latency to t-MDS/AML was 7 years (range 3–13 years). The diagnoses included t-AML ($n = 5$), acute promyelocytic leukaemia (APL, $n = 2$), refractory anaemia with excess blasts-1 (RAEB-1, $n = 3$), RAEB-2 ($n = 3$), chronic myelomonocytic leukaemia-2 (CMML-2, $n = 1$) and refractory cytopenia with multilineage dysplasia (RCMD, $n = 3$). Cytogenetic analysis showed normal karyotypes in two patients, t(15;17) in two patients with APL, monosomies/deletions involving chromosomes 5q and/or 7q (−5/5q–, −7/7q–) and other complex changes in 11 patients, and trisomies involving chromosomes 8, 9 and 21 in two patients. The median survival was 8 months (1–44 months) with or without treatment. Three patients were in complete remission after receiving allogeneic BMT (two patients with t-MDS) and treatment with arsenic trioxide (one patient with APL). One patient with RCMD was alive with disease.

Aberrant p15 gene methylation

Aberrant *p15* methylation was detected in 10 patients (59%) at the diagnosis of t-MDS/AML. Antecedent chemotherapy and diagnosis (t-MDS or t-AML) were unrelated to the occurrence of *p15* gene methylation. Cytogenetically, the *p15*-unmethylated group contained all the patients with normal karyoytpes ($n = 2$), t(15;17) ($n = 2$), and −5/5q– in the absence of −7/7q– ($n = 3$). However, *p15* methylation was significantly associated with −7/7q– ($P = 0{\cdot}002$). *p15* methylation had no influence on the median blast percentage and survival (Table I).

Serial analysis of p15 methylation

Only six patients had marrow samples available for testing before and/or after the diagnosis of t-MDS/AML. In three patients (patients 3, 7 and 15), aberrant *p15* methylation was first detected at 26, 4 and 17 months before the diagnosis of t-MDS (7, 5·5 and 2·5 years after chemotherapy) respectively. In patient 3, *p15* methylation was undetectable in the marrow after BMT (Fig 1).

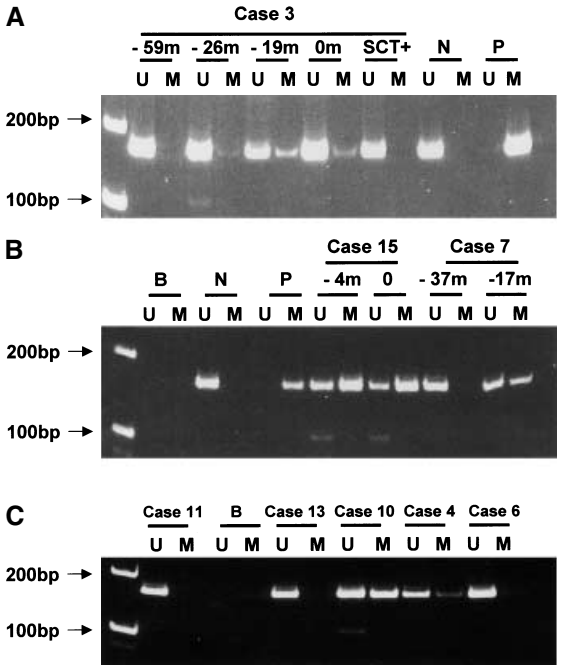

Fig 1. MSP for *p15* methylation. Primers were for M sequence respectively: forward M_F: 5′-TGA GGA TTT CGC GAC GCG TTC-3′, reverse M_R: 5′-CGT ACA ATA ACC GAA CGA CCG ATC G-3′; for U sequence: forward U_F: 5′-TGA GGA TTT TGT GAT GTG TTT-3′, reverse U_R: 5′-CAT ACA ATA ACC AAA CAA CCA ATC A-3′. PCR products for both the M and U sequences were 151 bp in size. B: reagent blank; N: normal donor DNA; P: positive control with universally methylated DNA. (A) In patient 3, *p15* methylation was first detected (weakly) at 26 months (−26 m) before t-MDS/AML was diagnosed. It disappeared after stem cell transplantation (SCT+). (B) In patient 15, *p15* methylation was first detected at 4 months (−4 m) before t-MDS/AML was diagnosed. In patient 7, *p15* methylation was detected at 17 months (−17 m) before t-MDS/AML was diagnosed, but not at 37 months (−37 m). (C) Patients 6, 11 and 13 were negative for *p15* methylation, whereas patients 4 and 10 were positive.

DISCUSSION

t-MDS/AML is an important long-term complication after chemotherapy/radiotherapy (Pedersen-Bjergaard *et al,* 2000). Apart from karyotypic aberrations, other genetic alterations, particularly aberrant methylation leading to gene silencing, are not well defined in t-MDS/AML. Furthermore, the similarities/differences in genetic alterations in t-MDS/AML and *de novo* MDS/AML with similar cytogenetic changes are also largely unknown.

Our results showed that *p15* methylation occurred frequently in t-MDS/AML. In the comparison of t-MDS/AML with *de novo* MDS/AML, there were a number of interesting observations. *p15* methylation was significantly associated with −7/7q– in t-MDS/AML. Previous studies have shown that t-MDS/AML with −7/7q– generally has fewer additional cytogenetic aberrations and is often associated with mutations of the *RAS* gene (Pedersen-Bjergaard *et al,* 2002). Our observations suggest that *p15* methylation might be another step, in addition to −7/7q–, during the pathogenesis of t-MDS/AML. However, our findings were observed in a relatively small number of patients and will need to be validated in studies of larger numbers of patients with both *de novo* as well as t-MDS/AML.

Although the frequency of *p15* methylation in t-MDS/AML in this study was similar to *de novo* MDS (34–50%) (Uchida *et al,* 1997; Tien *et al,* 2001) and AML (54–93%) (Wong *et al,* 2000; Chim *et al,* 2001), pattern differences were observed. In the present study, *p15* methylation was absent in both patients with t-APL, which contrasted with the high frequency of *p15* methylation (73–100%) reported in *de novo* APL (Wong *et al,* 2000; Chim *et al,* 2001). Furthermore, blast percentage and evolution from MDS to AML were unrelated to *p15* methylation in t-MDS/AML, which was different from *de novo* MDS (Quesnel *et al,* 1998), where an increase in blasts and leukaemic evolution were associated with *p15* methylation. The reasons for these differences are not clear and

will need to be further investigated in larger numbers of patients.

Finally, aberrant *p15* methylation might precede t-MDS/AML by several years, implying that this was an early event during disease evolution. Similar observations have not been made in *de novo* MDS/AML, as antecedent marrow samples are rarely available. With the high sensitivity of MSP (10^{-3}–10^{-4}), these observations suggested that examination for *p15* methylation might be useful in detecting the early emergence of t-MDS/AML clones in selected high-risk patients. Further prospective studies will be required to verify whether earlier detection of t-MDS/AML may impact on treatment results and prognosis in these patients.

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