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Frequent Low Level Expression in Ewing Sarcoma Family Tumors and Widespread Absence of the Metastasis Suppressor KAI1/CD82 in Neuroblastoma

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ABSTRACT

The transmembrane 4 superfamily member KAI1/CD82, a metastasis suppressor, is correlated inversely with the progression and invasion of several tumors. It is capable of inhibiting metastasis without affecting tumorigenicity *per se*. KAI1/CD82 expression is down-regulated in the progression of common solid epithelial tumors of adulthood. Mutation of p53 is suggested to be involved in the modulation of KAI1. As little is known about its expression and possible prognostic impact in pediatric tumors, we investigated KAI1/CD82 expression in cell lines and primary tumor samples from pediatric tumors of neuroectodermal origin, neuroblastoma and Ewing's sarcoma family tumor. Twenty-four of 29 Ewing's sarcoma family tumor cell lines, independent of p53 status, showed *KAI1* mRNA positivity by reverse transcription-PCR analysis in contrast to zero of eight neuroblastoma cell lines. Among 13 primary Ewing's sarcoma family tumor samples from patients with different disease extension, *KAI1* mRNA expression was low as detected by reverse transcription-PCR. Twenty of 30 primary neuroblastoma specimens were *KAI1*-negative by immunofluorescence analysis whereas the remaining 10 gave weak to moderate staining patterns. There was no apparent correlation of *KAI1* expression with any clinical or genetic features of the patients whose tumor samples were studied. Consequently, *KAI1* may not be of prognostic relevance in this group of tumors although there may be some role for *KAI1* modulation in the biology of these neuroectodermal tumors. **(*Pediatr Res* 52: 279–285, 2002)**

Abbreviations
EFT, Ewing sarcoma family tumors
NB, neuroblastoma
RT-PCR, reverse transcription-PCR
SSCP, single-strand conformational polymorphism
TM4SF, transmembrane 4 superfamily

A major challenge in oncology is to predict metastatic potential of tumor cells. The genetic events controlling metastasis are presently poorly understood (1). Metastasis suppressor genes, which are functionally inactivated as tumor cells acquire metastatic ability, may have significant prognostic value. Several metastasis suppressor genes have been mapped, but only few cloned to date (2, 3). KAI1 protein is a member of the TM4SF (4), which are cell membrane proteins. Many of the TM4SF members, including KAI1, are CD antigens present on the surface of leukocytes (5), and KAI1 is designated CD82 by the clusters of differentiation (CD) nomenclature (6). At least three members of the TM4SF family have been implicated in metastasis, including CD9/MRP-1, (7) CD63/ME491 (8), and KAI1/CD82 (9). KAI1 (CD82) contains three potential N-linked glycosylation sites and is a glycoprotein (9–12). KAI1 and other TM4SF members have been shown to bind to each other (5), integrins (13, 14), and E-cadherin (15). Physical association with other molecules has been reported for several TM4SF members (16, 17), which may underline a mechanism for their diversity of function.

KAI1 mRNA is ubiquitously expressed, with abundant levels in the surface of the major epithelial tissues, including lung, breast, prostate, and the gastrointestinal tract (18, 19). The physiologic roles of TM4SF proteins are largely unknown, but the proteins are implicated in signal transduction (20, 21), cell–cell interactions (12, 22, 23), cell–extracellular matrix interactions (24), T-cell activation, and development (4, 11, 25). These putative functions are consistent with a role in malignancy. The potential significance of *KAI1* as a metastasis suppressor gene was initially

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implicated in three human cancers; prostatic, non–small-cell lung, and pancreatic carcinomas. Advanced prostate tumors show decreased KAI1 expression relative to normal prostate and benign prostatic hyperplasia, and the down-regulation does not involve mutation or allelic loss of *KAI1* (3). It has also been reported that both Gleason grade and clinical stage of prostate cancers have an inverse correlation to the percentage of KAI1-positive cancer cells (26). Higher Gleason grade prostate cancers or clinically advanced stages exhibit reduced levels of KAI1 expression. In non–small-cell lung cancers, the survival rate of patients with KAI1-postive cancer was significantly higher than the survival rate of patients with KAI1-negative cancer (27). Additionally, advanced pancreatic tumor stages, in which metastases are present, have reduced KAI1 mRNA levels relative to earlier pancreatic tumor stages (28). A recent study revealed that wild-type tumor suppressor *p53* can directly activate *KAI1/CD82* gene expression (29). On the contrary, immunohistochemical analysis of tumor tissue samples from prostate cancer patients showed that among p53-negative tumor samples, 39% turned out to be KAI1/CD82-positive, pointing to the possibility that KAI1/CD82 expression in cancer cells is regulated by other pathways *in vivo* (29). *KAI1* has also been shown to be involved in non–small-cell lung cancer, bladder cancer, breast cancer, and gastric cancer (18, 27, 28, 30, 31). So far, studies have focused mainly on epithelial-derived tumors of adults. We now report the first study on *KAI1* expression in the progression of two different pediatric tumors of neuroectodermal origin, EFT and NB.

EFT is a group of small-round-cell tumors that affects bone and soft tissues in children and young adults. EFT patients can be assigned to one of two different risk groups depending on the extent of the disease at diagnosis. Although approximately 60% of patients with localized disease can be cured by modern multimodal treatment regimens, patients with metastases are at an 80% risk of succumbing to the disease (32, 33). The genes responsible for metastasis in these tumors have not been well characterized. We have previously studied a candidate antimetastasis gene in EFT, *nm23-H1* (34), but no correlation with prognosis or any pathophysiologic state could be established. NB is the most frequent solid tumor in children, forming approximately 8% of all pediatric tumors (35). The biologic diversity of neuroblastic tumors is vast and includes the phenomenon of complete, spontaneous regression (36) and maturation (37), even of disseminated tumors. In localized disease, regression processes are not only restricted to stage 1 or 2 disease, but may also, even if only rarely, be seen in patients with stage 3 disease (38). Besides these genetically and prognostically well-defined groups of NB (39), there are still some subsets of tumors for which further prognostic assessments would be needed. The purpose of this study was to investigate *KAI1* expression in EFT samples and in human NB for possible impact on prognosis.

METHODS

Tissue samples, cell lines, and culture conditions. The primary EFT tumors described in this report were diagnosed as Ewing's sarcoma or peripheral primitive neuroectodermal tumor according to the criteria of the Cooperative Ewing's Sarcoma Study (CESS). The presence of diagnostic *EWS-ets* gene fusions has been confirmed in every case by routine RT-PCR. The frozen NB specimens used for the immunofluorescence were obtained from the collection in the Children's Cancer Research institute. The study was approved by the local institutional ethics committee, and informed consent was obtained from the parents.

Cell lines used were routinely maintained in RPMI 1640 (Invitrogen, Carlsbad, CA, U.S.A.) supplemented with 10% fetal bovine serum, penicillin (0.1 mg/mL), and streptomycin (0.1 mg/mL) in 5% CO_2 at 37°C. The cell line SK-N-MC was a generous gift from Dr. J. Biedler (Memorial Sloan-Kettering Cancer Center, New York, NY, U.S.A.), WE-68, WE-M1-68, WE-M2-68, and VH64 were kindly provided by Dr. F. van Valen (Department of Pediatrics, University of Münster, Münster, Germany), TC-252 was a gift of Dr. T. Triche (Department of Pathology, Children's Hospital, Los Angeles, CA, U.S.A.), and A673, RDES, SK-ES1, LAN-1, and LAN-5 were obtained from the American Type Culture Collection (Rockville, MD, U.S.A.). Cell line IARC-EW2 was kindly supplied by Dr. G.M. Lenoir (International Agency for Research on Cancer, Lyon, France) and Vi856 by Otto Majdic (Institute for Immunology, Vienna, Austria). STA-ET-1, STA-ET-3, STA-ET-9, STA-ET-6, STA-ET-7, STA-ET-8, STA-NB-1, STA-NB-4, STA-NB-6, STA-NB-8, and STA-NB-10 are cell lines established in our institute.

RT-PCR and Northern blot analysis. First-strand cDNA synthesis was performed with 5 μg of total RNA using random hexamer oligodeoxyribonucleotides (Pharmacia, Uppsala, Sweden) with standard procedures. On the basis of the nucleotide sequence of *KAI1/CD82* (9), 5'-AGTCCTCCCTGCTGCTGTGTG-3' was used as the sense primer and 5'-TCAGTCAGGGTGGGCAAGAGG-3' as the antisense primer to amplify a 1030-bp fragment representing the coding sequence of the *KAI1* gene. Briefly, 5 μL of cDNA was used for amplification with 0.2 U DyNAzyme DNA polymerase (Finnzymes, Espoo, Finland) in 50 mM KCl, 1.5 mM $MgCl_2$, 10 mM Tris-HCl (pH 9.0), 0.1% Triton X-100, and 0.2 mM of each of the four dNTPs in a 50-μL final reaction volume. The reaction mixture was subjected to 30 PCR amplification cycles of 40 s at 94°C, 60 s at 60°C, and 60 s at 72°C, preceded by a primary denaturation step of 10 min at 94°C and followed by a final extension step of 7 min at 72°C after the last cycle in a thermal cycler (Biometra, Göttingen, Germany). β-actin DNA amplification with the primers *Act1*, 5'-CTTCCTGGGCATGGAGCTC-3' and *Act2*, 5'-CGCTCAGGAGGAGCAATGAT-3', yielding a 210-bp product, was used as the internal PCR control under the same conditions. Water blanks were included routinely to control for possible DNA carryover and contamination. The amplified DNA samples were run on a 1% agarose gel, and bands were visualized with ethidium bromide and photographed. *p53* mutation was measured by PCR-SSCP analysis of genomic DNA and direct sequencing of affected genes (40).

For Northern analysis, 10 μg of each RNA sample per lane, fractionated through a 1.2% agarose/1.8 M formaldehyde gel, was probed with a ^{32}P-labeled full-length *KAI1* cDNA. Quality and comparable loading of RNA samples were confirmed by including ethidium bromide in the gels and by rehybridizing blots to an 18S rDNA probe. After autoradiography, the filters were then exposed overnight to a Packard screen and scanned

at 50-μm resolution in a phosphorimager instrument for quantification (Cyclone Instrument; Packard, Meriden, CT, U.S.A.).

Immunoblot analysis. The Western blot protocol was essentially based on previously reported methods with some modifications (10). Briefly, cell monolayers were homogenized with extraction/lysis buffer [10 mM Tris-HCl (pH 8.0), 150 mM NaCl, 3 mM $MgCl_2$, 0.5% NP40, 2 mM phenylmethylsulfonyl fluoride, 1 μg/mL leupeptin, and 1 μg/mL aprotinin] after centrifugation at 4°C for 5 min at 1200 rpm. The lysates were then centrifuged at 14,000 rpm at 4°C for 10 min. Approximately 20 μg of cellular protein was size fractionated by 10% SDS-PAGE, and Western blots were incubated with anti-KAI1 C33 hybridoma supernatant (10) at 1:100 dilution. To ensure equal loading of protein, control experiments were performed with an actin MAb (ICN Biomedicals, Inc. Aurora, OH, U.S.A.).

Immunohistology. KAI1 expression was analyzed on frozen tissue sections of 30 neuroblastic tumor specimens with different genetic make-ups by immunohistology. Stroma-poor areas were investigated from 26 tumors and ganglioneuromatous areas from four tumors. In two cases, lymph node metastases were investigated and not primary tumor material. Twenty-seven tumor specimens were obtained at the time of diagnosis, three after administration of cytotoxic therapy. Briefly, 6-μm frozen sections were fixed with 4% paraformaldehyde (Merck, Darmstadt, Germany) for 10 min at 4°C and air-dried. After a washing step, fixed sections were incubated with a mouse anti-KAI1 C33 MAb at a dilution of 1:100 for 1 h. Visualization of bound antibodies was performed by using an FITC-labeled rabbit anti-mouse antibody. Nuclei were counterstained with 4'-6-diamidino-2-phenylindole-2HCl (DAPI). The staining pattern of the KAI1 protein in the neuroblastic tumors was judged independently by two observers blind to the *KAI1* mRNA/protein data.

RESULTS

KAI1 *mRNA expression in EFT and NB cells.* We initially examined whether *KAI1* mRNA expression varied among the various EFT and NB tumor samples by analyzing respective cell lines using RT-PCR. *KAI1* transcript levels were detected in 24 of 29 EFT and zero of eight NB cell lines examined. Figure 1 shows variable levels of *KAI1* mRNA expression in a representative series of EFT cell lines derived from different tumor stages at diagnosis (Table 1). NB cell lines established from primary tumors representing different stages of the disease showed either very weak or no measurable *KAI1* mRNA expression (Fig. 1). RT-PCR analysis also showed variably weak or absent levels of *KAI1* mRNA in primary EFT samples analyzed (in comparison to lymphocytes) irrespective of the metastatic status at diagnosis. Also, a heterogenous expression pattern among the different tumor samples was observed with most tumors showing undetectable or relatively faint *KAI1* mRNA expression (data not shown). No correlation of *KAI1* expression to the metastasis state in the primary tumors could be found.

To further evaluate the expression of *KAI1* message in several EFT and NB cell lines, we also performed Northern blot analyses using a full-length *KAI1* cDNA fragment as a probe. Steady-state transcript levels of *KAI1* was quantified by phosphorimager analysis and normalized by comparison with the hybridization signal obtained with an 18S rDNA probe. A summary of the results is provided in Table 1. Although there were some differences in the level of expression among those

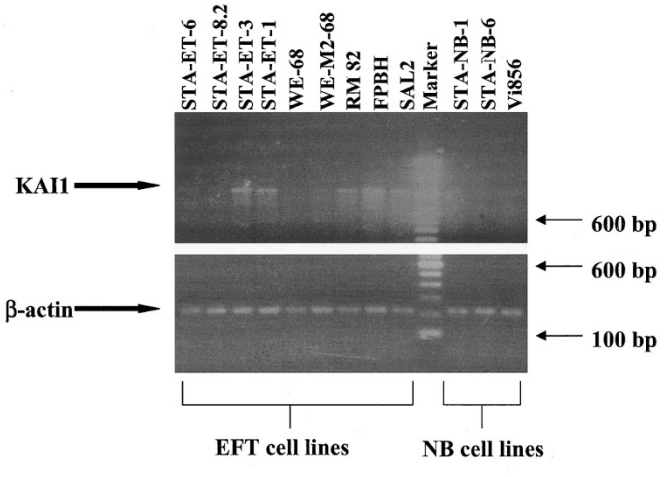

Figure 1. Representative photographs from ethidium bromide–stained gel of RT-PCR–amplified 1031-bp *KAI1* cDNA and 210-bp β-actin cDNA. EFT cell lines, NB cell lines, and size marker are indicated for both *KAI1* and β-actin PCR-amplified products.

Table 1. *p53 status and KAI1 mRNA levels in EFT cell lines*

Cell line	Fusion type*	Origin	*p53* Status	*KAI1* Expression†
STA-ET-1	(7/6) 1	Relapse	wt	1.8
STA-ET-2.1	(9/4)	1° tumor	mut	1
STA-ET-2.2	(9/4)	Metastasis	mut	1.2
STA-ET-3	(7/6) 1	Metastasis	wt	1.8
STA-ET-4	(7/6) 1	1° tumor	nd	1.6
STA-ET-5	n.d.	1° tumor	nd	0.8
STA-ET-6	(7/6) 1	Pleural effus.	wt	1.2
STA-ET-7.1	(7/5) 2	1° tumor	mut	0.8
STA-ET-7.2	(7/5) 2	Pleural effus.	mut	1.2
STA-ET-7.3	(7/5) 2	Metastasis	mut	1.4
STA-ET-8.1	(7/5) 2	1° tumor	mut	1
STA-ET-8.2	(7/5) 2	Pleural effus.	nd	0.9
STA-ET-9	(7/6) 2	1° tumor	wt	1.2
STA-ET-10	FEV	1° tumor	wt	1.6
STA-ET-11	ERG	1° tumor	wt	1.6
STA-ET-12	(7/5) 2	1° tumor	wt	nd
SAL1	(7/6) 1	1° tumor	wt	nd
SAL2	(7/6) 1	Relapse	wt	nd
A673	(7/6) 1	nd	mut	1.6
RDES	(7/5) 2	1° tumor	mut	1.8
EW2(D)	(7/5) 2	Blood	mut	1.4
TC252	(7/6) 1	Metastasis	wt	1
VH64	(7/5) 2	Metastasis	wt	1
SK-N-MC	(7/6) 1	Metastasis	mut	1.2
SK-ES1	(7/5) 2	1° tumor	mut	0.8
WE 68	(7/6) 1	1° tumor	wt	1
WE-M1-68	(7/6) 1	Metastasis	wt	1
WE-M2-68	(7/6) 1	Metastasis	wt	1.2
RM82	ERG	1° tumor	mut	1.4

* (7/6) 1, EWS exon 7 and FLI1 exon 6 fusion, type 1; (7/5) 2, EWS exon 7 and FLI1 exon 6 fusion, type 2; (FEV), EWS and FEV fusion; (ERG), EWS and ERG fusion.

† Relative *KAI1* expression data, normalized to 18S rDNA control, obtained by phosphorimager analysis of Northern blots.

Abbreviations used: wt, wild type; mut, mutation; nd, not determined; 1° tumor, primary tumor; Pleural effus., pleural effusion.

cell lines studied, we observed *KAI1* mRNA of about 2.4 kb in most of the EFT cell lines (Fig. 2) but no or relatively weak *KAI1* transcript was detected in the NB cell lines studied (Fig. 2). The variability in the *KAI1* mRNA expression levels could not be correlated with the *p53* mutation status in all the EFT cell lines (Table1). The Northern data corroborated the RT-PCR results.

KAI1 protein levels in EFT and NB cell lines. To confirm the expression of *KAI1* gene at the protein level, all the EFT and NB cell lines studied for mRNA expression were also examined for their KAI1 protein content by immunoblot analysis. KAI1 protein from the EFT cell lines migrated between M_r 30,000 and M_r 60,000 and appeared as smear owing to the presence of glycosylation variants of different molecular weights (Fig. 3). No KAI1 expression was observed in the NB cell lines analyzed (Fig. 3) in concordance with the mRNA data. The level of KAI1 protein in two EFT cell lines, STA-ET-1 and STA-ET-3, was comparable to that in the MOLT-4 cell line, which has been reported to express high levels of KAI1 protein (10). KAI1 expression size in the MOLT-4 cell line, which served as the positive control, varied from M_r 30,000 to more than M_r 66,000 in molecular weight, whereas no expression was observed in the NIH 3T3 cell line, which served as our negative control (Fig. 3). The glycosylation pattern among the different EFT cell lines varied considerably, with some showing discrete bands, instead of the normal smear, higher than 28 kD, which is the size of the predicted molecular weight of KAI1 on the basis of its amino acid sequence (9). A representative Western blot for actin protein (Fig. 3, *bottom*) showed consistent loading of protein from the different cell lines. We also investigated the relationship of KAI1 expression and another TM4SF protein, CD81 (TAPA 1) in some of the cell lines. We observed that although KAI1 showed heterogenous expression, CD81 was homogeneously expressed in the cell lines analyzed (data not shown). KAI1 protein expression as examined by Western blot analysis was also consistent with *KAI1* RNA expression as measured by RT-PCR analysis in all cell lines studied with the exception of the STA-ET-6 cell line in which, although considerable level of mRNA was expressed, no protein was detected.

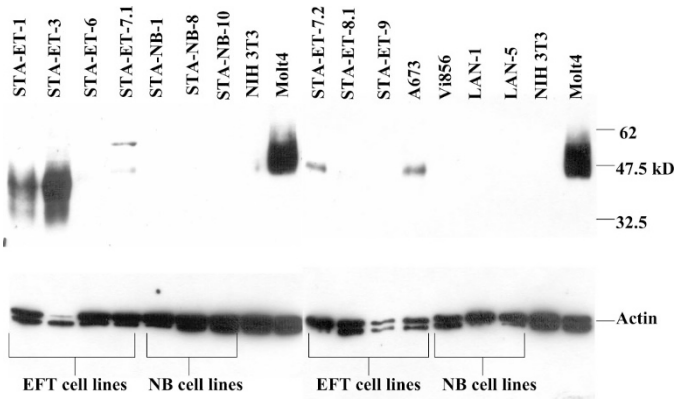

Figure 3. Western blot analysis of KAI1 expression in representative EFT and NB cell lines as well as a positive (MOLT-4) and a negative (NIH 3T3) control cell lines. Protein cell lysates were prepared from cell lines, size-fractionated by SDS-PAGE, transferred to nitrocellulose membranes, incubated with anti-KAI1 C33 or actin antibodies, and exposed to film. Size markers in kD are noted.

KAI1 expression does not correlate with any genetic markers of NB tumors. Inasmuch as *KAI1* expression was relatively undetectable in all the NB cell lines analyzed, we decided to check for possible expression in primary NB tumor samples by immunofluorescence analysis. Twenty of the 30 tumor specimens analyzed did not show any reaction with the *KAI1* C33 antibody (Fig. 4*A*, Table 2). Ganglioneuromatous areas showed either no reaction (two cases, Fig. 4*B*) or positive reactions in differentiating cells (one case) and some ganglionic-like cells (one case after therapy). Stroma-poor areas of eight tumors showed either a weak positivity in single areas (two cases) or a weak to moderate positivity in the majority of the tumor cells (six cases, Fig. 4, *C* and *D*, one after therapy). Lymphocytes always showed strong reactions, unequivocally exceeding the positivity found in the tumor cells (Fig. 4, *A*, *E*, and *F*) and so served as our internal positive control. No correlation was found between *KAI1* expression and genetic markers of the tumor, such as *MYCN* amplification/gain, chromosome 1p36.3 deletions/imbalances (Table 2) or gains of the long arm of chromosome 17 (data not shown).

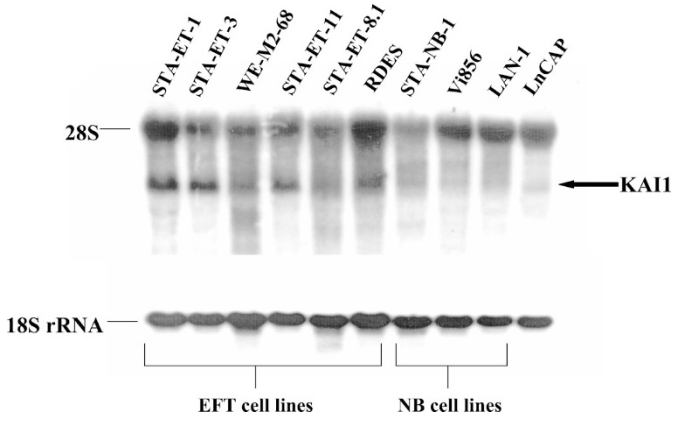

Figure 2. Representative Northern blot analysis of *KAI1* mRNA expression in various EFT and NB cell lines. Although variable expression levels were observed in the EFT cell lines, no *KAI1* transcript was detectable in the NB cell lines, and *KAI1* probe cross-reacted with 28s. The filters were rehybridized to an 18S rDNA probe to check quality and loading of RNA.

DISCUSSION

Among all genetic aberrations, inactivation of metastasis suppressor genes is one important factor contributing to the formation of tumor metastasis. *KAI1* expression has previously been demonstrated to inversely correlate with the metastatic potential and consequently with prognosis of several epithelial neoplasms in adults. This is the first report evaluating *KAI1* expression in pediatric tumors. We have focused on two neuroectodermal neoplasms, NB and EFT. Although no or very low level expression of *KAI1* was generally observed in NB, variable but, when compared with bone marrow cells, mostly low positivity was observed in EFT. The most comprehensive *KAI1* analysis was performed on cell lines. Although two EFT cell lines showed KAI1 protein levels comparable to that of MOLT-4, the overall results obtained were in line with those of

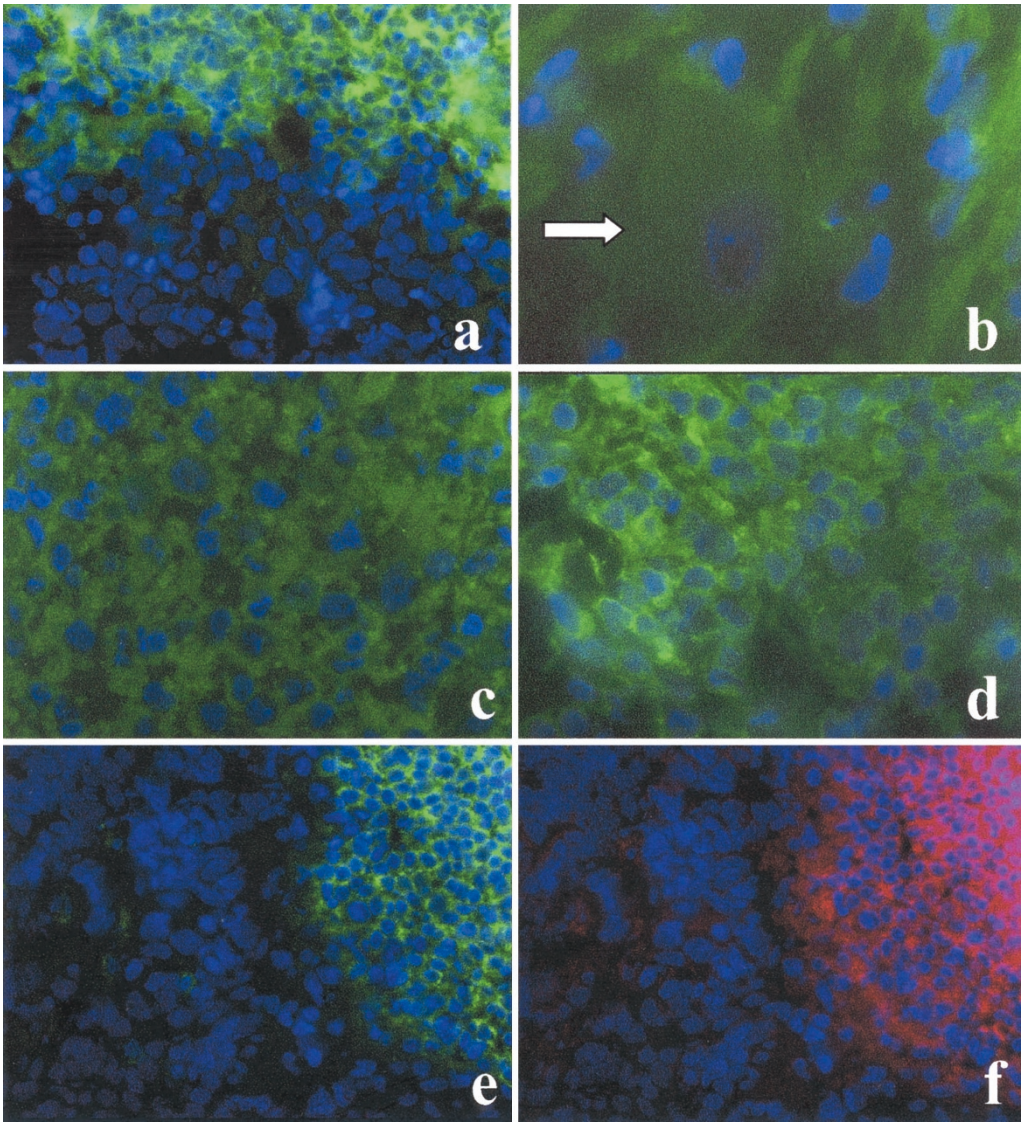

Figure 4. Immunofluorescence detection of *KAI1* in samples from patients with different stages of NB. Frozen sections were incubated with the C33 MAb. *A*, *KAI1*-negative tumor cells (*blue*) surrounded by *KAI1*-positive lymphocytes (*green*). *B*, ganglioneuromatous tissue with *KAI1*-negative neoplastic ganglionic cells. *Arrow* points to a ganglionic cell. *C*, stroma-poor NB with a weak *KAI1* positivity. *D*, stroma-poor NB with a weak to moderate *KAI1* expression. *E* and *F*, lymphatic tissue was used as an internal positive control (double staining was performed with a CD45 antibody and an FITC-conjugated secondary antibody, shown in *E*, and KAI1 highlighted with a TRITC-conjugated secondary antibody, given in *F*). Magnification *A, C, D* = ×375; *B* = ×750.

White *et al.* (19), who analyzed KAI1 protein in normal and cancer cells of a variety of tissues and observed that KAI1 protein was specifically down-regulated in most of the cancer cell lines analyzed. Cell lines may in fact represent a group of patients with bad prognosis because establishment of *in vitro* cell cultures has been shown to select for secondary genetic aberrations, including *p53* mutations and *INK4A* deletions in EFT (41), and disruption of *p53* function has been correlated with KAI1 deficiency in some malignancies, including prostate tumors (29). In our study, however, RT-PCR analysis of a small number of primary EFT and immunohistologic analysis of a series of primary NB corroborated the cell line results giving no indication of a stage-dependent expression of KAI1 in these tumors. Also, for EFT no correlation with the *p53* status was seen. In addition, using an EFT cell line carrying a conditional *p53* mutant (p53val138) no changes in *KAI1* expression were noted on *p53* induction (data not shown). Thus we were unable to confirm a correlation between *KAI1* expression and *p53* status as has been controversially discussed in the literature before (42). In our series, low *KAI1* expression was already detected on the RNA level. Although we have not tested for the genomic status of the *KAI1* gene in NB and EFT, evidence so far obtained from other tumors with reduced or absent *KAI1* expression suggests that neither loss of heterozygosity at the *KAI1* locus (43, 44) nor the presence of mutations within the *KAI1* gene (3, 45, 46) nor hypermethylation of a CpG island within the *KAI1* promoter (42) appears likely to be responsible. The presence of an extra-large intron 1 and non-coding exons at the 5'-region of the *KAI1* gene suggests that the regulation of *KAI1* gene expression may not be simple (47).

EFT are characterized by expression of an *EWS-ets* gene rearrangement of variable architecture. For localized disease

Table 2. *Summary of NB patients characteristics and KAI1 expression*

Patient No.	Sex	Age (y)	Stage	*MYCN* Amplif.	Deletion 1p	*KAI1* Expression
1	male	1	4	negative	negative	+
2	female	1.5	4	negative	?	−
3	na	na	na	na	na	−
4	male	0.9	4s	negative	negative	+
5	male	1	2B	negative	positive	+
6	male	3.9	4	negative	imbalance	−
7	female	0.5	2R	negative	imbalance	−
8	female	0.9	3R	negative	negative	−
9	male	0.9	4s	pos. fokal	positive	−
10	female	1	2R	negative	negative	±
11	male	1.5	4	positive	imbalance	−
12	female	1.3	2A	negative	negative	−
13	female	5.7	4	negative	negative	−
14	male	0.9	1	negative	negative	−
15	female	0.2	4s	negative	negative	−
16	female	2.6	na	positive	positive	−
17	male	2.3	4	positive	positive	−
18	male	0.0	na	negative	negative	−
19	female	na	na	negative	imbalance	−
20	female	0.1	1	negative	imbalance	−
21	male	0.4	4s	negative	negative	−
22	male	0.4	1	negative	negative	−
23	female	2.5	na	negative	negative	−
24	female	1.3	4	negative	negative	+
25	male	0.1	1	negative	negative	−
26	female	1.3	na	negative	negative	+
27	male	1	3U	positive	negative	±
28	female	1.2	1	negative	negative	−
29	male	1	3R	negative	negative	+
30	male	0.9	3U	negative	negative	+

Abbreviations used: −, no expression; ±, weak positivity in single cells; +, weak to moderate positivity; na, not available.

the two main fusion types *EWS-FLI1* type 1 and type 2 appear to be associated with distinct clinical outcomes (48–51). In addition, patients who present with clinically overt metastatic disease at diagnosis generally have an adverse prognosis. In our series of EFT cell lines and primary tumors, variation in *KAI1* expression correlated neither with *EWS-FLI1* fusion type nor with the stage of the disease at which the tumor material was collected. It should be noted, however, that in primary EFT, *KAI1* expression, if present, was low. Because in the prechemotherapy era, when EFT were treated solely by surgery and irradiation, most patients succumbed to distant relapse, EFT may be envisaged as a generally metastasizing disease. Low *KAI1* expression may therefore reflect a common metastatic potential in this disease. Our results are generally in concordance with KAI1 studies on bone and soft tissue tumors in which no correlation was found between metastasis of osteosarcoma cells to the lungs and loss of *KAI1/CD82* (52).

By contrast, the spectrum of NB presentation reaches from highly aggressive metastasizing disease to a spontaneously regressing or maturing localized tumor. Several genetic traits are considered to be associated with distinct clinical outcome in NB, including ploidy, *MYCN* amplification, chromosome 1p36 deletions or imbalances, and gain of chromosome 17q. Our series of NB tumors comprised representative material from all disease stages and different genetic make-ups (Table 2). However, *KAI1* expression was found to be uniformly absent or very weak to moderate in 20 and 10 of 30 tumors, respectively, with no correlation to the extent of the disease, any genetic trait, or clinical outcome.

In summary, our data indicate that in contrast to a growing list of cancers in which down-regulation of *KAI1* is associated with tumor progression, *KAI1* expression cannot serve as a prognostically useful marker in NB and EFT. However, these results do not exclude a role for *KAI1* down-regulation in the biology of the disease. Also, our results clearly revealed a difference in the *KAI1* expression patterns between EFT and NB that may reflect differences in either the specific tumor biology or origin. Analysis of other pediatric malignancies of neuroectodermal and nonneuroectodermal histogenesis will contribute to a more general understanding of the role *KAI1* may play in children's cancer.

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