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Wilms' tumor gene (*WT1*) expression in lung cancer, colon cancer and glioblastoma cell lines compared to freshly isolated tumor specimens

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Abstract The Wilms' tumor gene (*WT1*) encodes a transcriptional regulator involved in growth and differentiation of various tissue types. A continuous over-expression of *WT1* was found in leukemic blasts, thus suggesting an oncogenic function. Solid cancer entities have also been described as expressing *WT1*. We systematically analyzed *WT1* expression in small-cell and non-small-cell lung cancer, colon cancer and glioblastoma patients and in the respective tumor cell lines. Using reverse transcription/polymerase chain reaction, we found *WT1* expression in glioblastoma (5 of 8), lung (5 of 11), and colon cancer (5 of 15) cell lines. While *WT1* was expressed in only 1 of 12 lung cancer and 1 of 5 glioblastoma specimens, it was not detected in colon cancer or macroscopically tumor-free colon and lung tissue. In addition, HT29 colon cancer cells showed a loss of *WT1* expression when grown to confluence or induced to differentiate by sodium butyrate. From this evidence, testing for *WT1* expression is not clinically relevant for colon cancer, lung cancer, or glioblastoma patients. *WT1* expression in cancer cell lines can probably be attributed to optimized in vitro growth conditions.

Key words Wilms' tumor gene expression · Colon cancer · Lung cancer · Glioblastoma · Cancer cell lines · Cancer specimen

Abbreviations *WT1* Wilms' tumor gene · *MNC* mono-nuclear cells · *EGFR* epidermal growth factor receptor · *SCLC* small-cell lung cancer · *NCLC* non-small-cell lung cancer

Introduction

The Wilms' tumor gene (*WT1*) encodes a zinc-finger-motif-containing transcription factor involved in the regulation of growth and differentiation of human cells. *WT1* has been mapped to chromosome 11p13. Since this genomic locus is frequently deleted in patients with hereditary Wilms' tumors (Call et al. 1990; Gessler et al. 1990; Rose et al. 1990), *WT1* is thought to act tumor-suppressively (Gessler et al. 1990). Subsequent research suggests that *WT1* also has oncogenic properties in the context of interaction with other transcription-factor genes like *p53* or *EGR-1* (Maheswaran et al. 1993; Menke et al. 1997). There is increasing evidence that continuous over-expression of *WT1* has a leukemogenic action (Inoue et al. 1997, 1994; Miwa et al. 1992; Miyagi et al. 1993; Yamagami et al. 1996). Furthermore, testing for *WT1* expression in acute leukemia patients has a clinical impact, since it may provide prognostic information indicating (Bergmant et al. 1997; Inoue et al. 1994) or predicting imminent relapse after cytoreductive treatment (Brieger et al. 1994; Inoue et al. 1996; Menssen et al. 1998).

Transcripts of *WT1* have been described in several other malignancies including ovarian cancer (Bruening et al. 1993; Viel et al. 1994), breast cancer (Silberstein et al. 1997), melanoma (Rodeck et al. 1994), mesothelioma (Amin et al. 1995; Langerak et al. 1995; Park et al. 1993; Walker et al. 1994), desmoplastic round-cell tumor (Ladanyi and Gerald 1994), and renal cell carcinoma (Campbell et al. 1998). Recently, *WT1* was found to be expressed in 82% of human solid cancer cell lines established from gastric carcinoma, colon cancer, lung cancer, breast cancer, and hepatocellular carcinoma (Oji et al. 1999). Moreover, the growth of

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three *WT1*-expressing cancer cell lines (AZ-521 gastric cancer, OS3 lung cancer, TYK-nu ovarian cancer) was suppressed by treatment with *WT1* antisense oligonucleotides. The authors thus concluded that *WT1* expression plays an essential role in promoting solid cancer growth (Oji et al. 1999).

Here, we address the question of whether these *in vitro* results can be translated to the clinical setting. We further investigated whether testing for *WT1* expression is clinically relevant for small-cell (SCLC) and non-small-cell lung cancer (NSCLC), colon cancer, and glioblastoma patients. Therefore, we analyzed cancer cell lines and freshly resected human tumor samples for *WT1* expression, using nested-primer reverse transcriptase/polymerase chain reaction (RT-PCR). To test the hypothesis that *WT1* expression correlates with rapidly growing undifferentiated cancer cells, we also studied *WT1* expression in HT29 colon cancer cells at different times after seeding and when the cells were induced to differentiate by butyric acid. HT29 cells display incomplete contact inhibition when grown to confluence *in vitro* and differentiate upon treatment with butyric acid (Augeron and Labois 1984; Nagel and Vallee 1995; Tanaka et al. 1989).

Materials and methods

Patients and cell lines

The cell lines of small-cell (HTB175, HTB173, HTB171, HTB120, HTB119) and non-small-cell lung cancer (HTB58, HTB57, HTB56, HTB53, CCL185, CCL 184), colon cancer (HTB29, COLO205, COLO320, CX1, CX2, CX-F94, DAN-G, HT23, SW48, SW620, SW707, SW948, SW480, LoVo, LS170), and glioblastoma (N66, N65, N64, N59, N39, N31, HTB14, HTB17) were obtained from ATCC (Rockville, Mass., USA) and propagated in the recommended media.

Lung cancer [SCLC ($n = 3$); NSCLC ($n = 9$)], colon cancer ($n = 6$), and glioblastoma ($n = 5$) specimens were obtained from the primary tumor site during operation (General Surgical and Neurosurgical Departments of the University Hospital Benjamin Franklin; Thoraco-Surgical Department of the Hospital Heckeshorn, Berlin, Germany). Under sterile conditions, tumor samples 0.5 cm in diameter (0.2 cm in diameter for glioblastoma) were taken and shock-frozen in liquid nitrogen. Samples from macroscopically tumor-free margins of the operative specimens were also processed accordingly. Normal brain tissue was not resected for ethical reasons and because of the neurosurgical procedure. Blood mononuclear cells (MNC) of healthy volunteers ($n = 5$, negative controls) and patients with B cell chronic lymphocytic leukemia ($n = 5$, negative controls) were analyzed for *WT1* gene expression, as were K562 and HL60 leukemic blasts (positive controls).

RT-PCR

Total RNA was prepared from 10^6 cells at the exponential growth phase of the cell lines using the RNazol extraction protocol (RNazol B, Wak-Chemie, Bad Homburg, Germany) as described elsewhere (Menssen et al. 1995). Frozen tumor samples were put in RNazol solution and disrupted in a 1-ml tissue homogenizer (neolab, Heidelberg). Total RNA was extracted as previously described. Reverse transcription was performed, using SuperScript RNase H⁻ reverse transcriptase (Gibco BRL, Gaithersburg, Md., USA). The integrity of the RNA of each sample was determined by amplifying

the *c-abl* protooncogene by RT-PCR (Menssen et al. 1995). A set of nested primers was used to amplify an approximately 480-bp DNA including the *WT1* zinc finger region (365 bp). The primer sequences for the first round of amplifications were the 20-mer 5'-ATG-TGC-GAC-GTG-TGC-CTG-GA-3', located outside the *WT1* zinc finger motif on exon 7 near the fusion site of exon 6 to exon 7, and the 12-mer oligo-(dT) 5'-TTT-TTT-TTT-TTT-3' (Sigma Chemie, Deisenhofen, Germany). The second round of amplifications was performed with a 22-mer, 5'-GAC-GTG-TGC-CTG-GAG-TAG-CCC-C-3', primer located on exon 7 outside of the *WT1* zinc finger motif and a 21-mer, 5'-GCT-GCC-TGG-GAC-ACT-GAA-CGG-3', primer located on exon 10, 17 bp upstream from the stop codon. A 5- μ l sample of RT reaction product was added to 95 μ l PCR mixture containing the first set of primers at 0.2 μ M concentration, 10 \times amplification buffer, 0.5 μ l Taq DNA polymerase (Pharmacia Biotech Europe, Freiburg, Germany), and dNTP at 200 μ M final concentration. A total of 56 cycles of the RT-PCR protocol were performed as described elsewhere (Menssen et al. 1995). The KTS⁺ and KTS⁻ *WT1* gene splicing variants were co-amplified. Owing to the small difference in their size, they could not be distinguished on 1.5% agarose gels. Amplification was performed in duplicate using reagents and Taq polymerase from Pharmacia Biotech Europe. PCR products were submitted to electrophoresis on ethidium-bromide-stained 1.5% agarose gels. *WT1* RT-PCR amplification products were characterized by restriction enzymes (*MscI*; *NsiI*, *HaeII*; Gibco, Paisley, Scotland).

Indirect immunofluorescence assay

To detect the nuclear *WT1* protein, 10^5 cells of each cell line were cytocentrifuged onto glass slides, air-dried, and stained by the mouse anti-(human *WT1*) monoclonal antibody H2 (Menssen et al. 1997b, Rauscher et al. 1998).

Cell growth assays and induction of differentiation

HT29 colon cancer, CCL185 non-small-cell lung cancer, and N59 glioblastoma cells were seeded into 96-well plates (5000 cells in 100 μ l medium/well). The medium was changed every 3 days. Cells were counted in an automated cell counter (Coulter Electronics GmbH, Krefeld, Germany). Total RNA was isolated and analyzed for *WT1* gene expression as previously described. Cell proliferation was also determined by measuring [³H]thymidine uptake. Cells were incubated with 20 μ l [³H]thymidine solution/well (1 μ Ci radioactivity/well) for 6 h at 37°C on days 2, 3, 5, and 8. Cells were washed and lysed; the incorporated radioactivity was measured (Betaplate 1205, LKB Wallac). For differentiation assays, HT29, CCL185, and N59 cells were seeded into 96-well plates. Simultaneously, cells were grown in 25-cm² flasks for standard *WT1* RT-PCR analysis (10^6 cells). The medium was supplemented with 5 mM sodium butyrate 48 h after seeding. Cells were counted, assayed for [³H]thymidine uptake and analyzed for *WT1* gene expression on days 2, 4, 6, and 8. The experiments were done in triplicate and repeated three times.

Results

We compared *WT1* expression in cancer cell lines and fresh tumor tissue to determine whether testing for *WT1* expression can be used to detect minimal residual disease in cancer patients, similar to acute leukemia patients. Three different tumor entities were selected for this approach on the basis of their frequency and clinical significance. Using RT-PCR, we found *WT1* expression in five out of 11 lung cancer (45%), 5 out of 15 colon cancer (33%), and 5 of 8 glioblastoma (63%) cell lines.

While 2 of 5 SCLC (40%) and 3 of 6 NSCLC cell lines (50%) expressed *WT1*, it was only detected in 1 of 12 lung cancer (NSCLC; 8%) and in 1 of 5 glioblastoma (20%) tumor specimens. No *WT1* expression was found in colon cancer specimens by nested primer RT-PCR. *WT1* was not expressed by macroscopically tumor-free lung or colon tissue or by the MNC of healthy volunteers or chronic lymphocytic leukemia patients. As expected, HL60 and K562 leukemic blasts strongly expressed *WT1*. *c-abl* gene expression was detected by RT-PCR in all samples analyzed (amplification fragment: 106 bp), thus proving the integrity of the RNA (Table 1). Restriction enzyme analysis of *WT1* PCR

products yielded fragments of the expected size (data not shown).

Indirect immunofluorescence with mAb H2, revealed WT1 protein in the nuclei of 1 (NSCLC HTB57) of 6 lung cancer cell lines. While two glioblastoma cell lines (N59, N64) showed typical homogeneous nuclear immunofluorescence, thus demonstrating translation of *WT1*, two others displayed no WT1 immunofluorescence (Fig. 1C). The WT1 protein was not detected in three of the colon cancer cell lines tested (Table 1). *WT1* expression was found by RT-PCR in all cell lines evidencing WT1 immunofluorescence. As expected, HL60 and K562 blasts showed a strong nuclear fluorescence,

Table 1 *WT1* gene expression in cancer cell lines and freshly obtained cancer specimens. Gene expression was detected by reverse transcriptase/polymerase chain reaction; WT1 protein was detected by indirect immunofluorescence using mAb H2; ND not done

Tissue	Cancer type	Detection of <i>WT1</i> gene expression (%)	Detection of WT1 nuclear protein	Detection of <i>c-abl</i> gene expression
Cell lines	SCLC (<i>n</i> = 5)	2/5 (40)	0/3	5/5
	HTB175	+	-	+
	HTB173	+	-	+
	HTB171	-	-	+
	HTB120	-	ND	+
	HTB119	-	ND	+
	NSCLC (<i>n</i> = 6)	3/6 (50)	1/3	6/6
	HTB57	+	+	+
	CCL185	+	-	+
	HTB53	+	ND	+
	HTB58	-	-	+
	HTB56	-	ND	+
	CCL184	-	ND	+
	Colon cancer (<i>n</i> = 15)	5/15 (33)	0/5	15/15
	HTB29	+	-	+
	SW620	+	-	+
	CX1	+	ND	+
	HT23	+	ND	+
	LS170	+	ND	+
	LoVo	-	-	+
	CX2	-	ND	+
	CX-F94	-	ND	+
	DAN-G	-	ND	+
	COLO205	-	ND	+
	COLO320	-	ND	+
	SW48	-	ND	+
	SW707	-	ND	+
	SW948	-	ND	+
	SW480	-	ND	+
	Glioblastoma (<i>n</i> = 8)	5/8 (63)	2/8	8/8
	N59	+	+	+
	N64	+	+	+
N39	+	-	+	
N31	+	ND	+	
HTB17	+	ND	+	
N65	-	-	+	
N66	-	ND	+	
HTB14	-	ND	+	
Cancer specimens	Lung cancer (<i>n</i> = 12)	1/12 (8)	ND	12/12
	Colon cancer (<i>n</i> = 6)	0/6 (0)	ND	6/6
	Glioblastoma (<i>n</i> = 5)	1/5 (20)	ND	5/5
Controls	Lung and colon cancer resectate margins (<i>n</i> = 36)	0/36 (0)	ND	36/36
	PBMNC (<i>n</i> = 5)	0/5 (0)	0/5	5/5
	B-CLL (<i>n</i> = 5)	0/5 (0)	0/5	5/5
	HL60 blasts	+	+	+
	K562 blasts	+	+	+

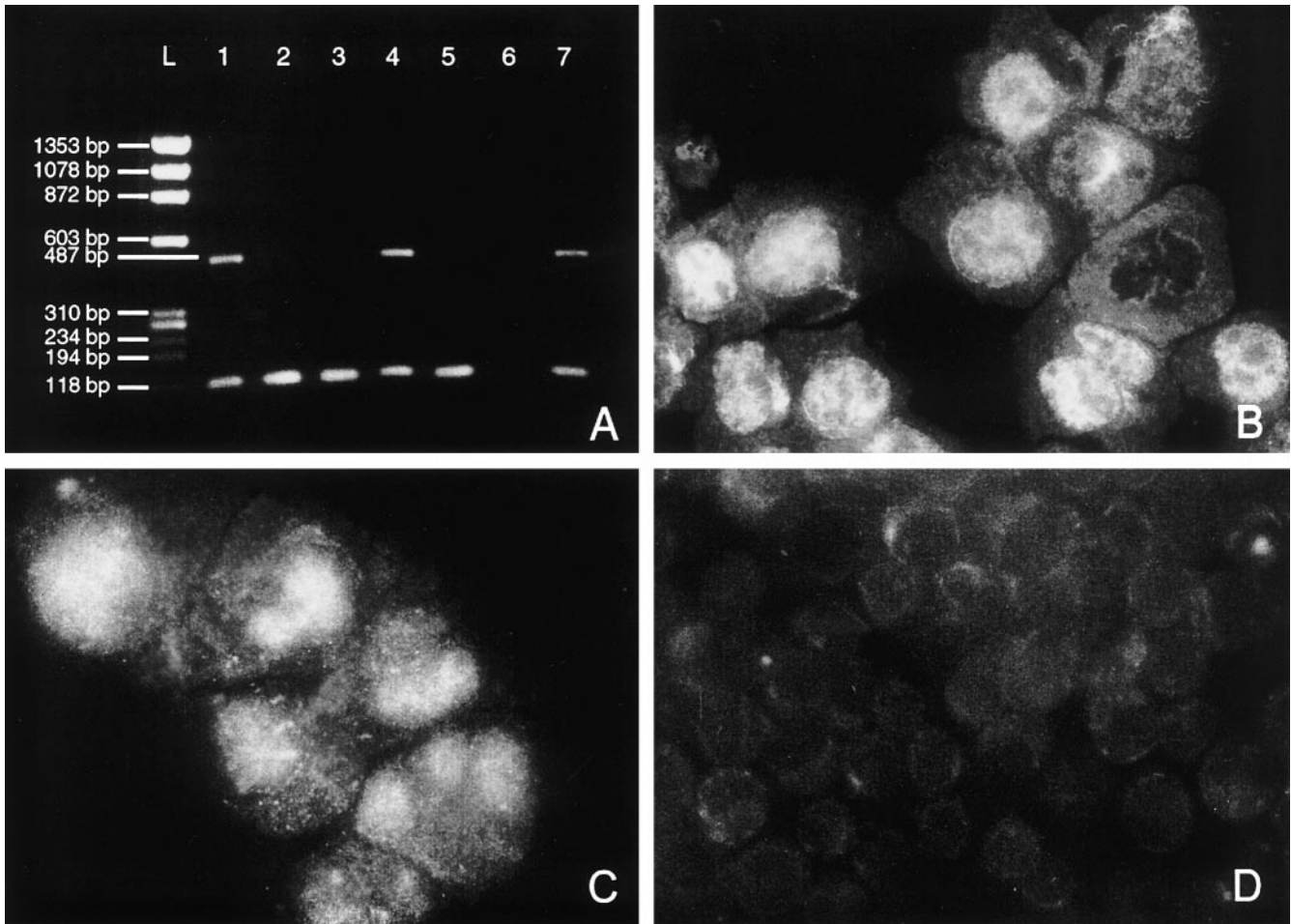


Fig. 1A–D Detection of *WT1* expression using reverse transcriptase/polymerase chain reaction (RT-PCR) and indirect immunofluorescence. **A** *WT1* RT-PCR and *c-abl* PCR amplicates from HTB57 non-small-cell lung cancer (NSCLC) cells, HTB119 small-cell lung cancer (SCLC) cells, fresh lung cancer tissue specimens (LC1, LC2, LC3), water control, and K562 blasts were electrophoresed on an ethidium-bromide-stained 1.5% agarose (lanes 1–7, respectively). *L* molecular mass markers. **B–D** When mAb H2 was used in indirect immunofluorescence, the WT1 nuclear protein was detectable in HL60 blasts (positive control, **B**) and HTB57 NSCLC cells (**C**), but not in B-CLL cells (negative control, **D**)

whereas WT1 immunofluorescence could not be detected in blood MNC from normal volunteers and B cell chronic lymphocytic leukemia patients (Table 1, Fig. 1B–D).

Furthermore, we analyzed HT29 colon cancer cells for *WT1* expression when they were grown to confluence or induced to differentiate by butyric acid. Five days after seeding into microtiter plates, HT29 cells repeatedly grew to form a confluent monolayer. Upon reaching confluence, HT29 cell growth was substantially reduced (plateau-like growth phase; Fig. 2A), and there was a highly significant reduction in [^3H]thymidine incorporation into the cells (Fig. 2B). No *WT1* expression could be detected in HT29 cells at that time or thereafter by RT-PCR. Trypan blue staining revealed that only

5%–10% of the HT29 cells had died at confluence (day 5; data not shown). However, HT29 cells grew rapidly 3 days after seeding, and *WT1* expression was detected (Fig. 2A, B). Even 8 days after seeding, HT29 cells did not reach confluence when grown in medium containing sodium butyrate to induce enteric differentiation. Beginning 2 days after propagation in sodium-butyrate-supplemented medium, HT29 cells displayed intracellular vesicles and a pseudo-acinous growth pattern (groupings of five to ten cells around circular empty spaces) as typical morphological features of enteric differentiation (data not shown). When grown in sodium-butyrate-containing medium, HT29 cells expressed *WT1* on days 2 and 4, while no *WT1* expression was detected in differentiation-induced HT29 cells on days 6 and 8 (Fig. 2C). Trypan blue staining demonstrated that the majority of HT29 cells were viable throughout the experiment. Differentiation-induced HT29 cells did not restart growth when transferred into regular growth medium on days 6 or 8, and *WT1* expression remained undetectable (data not shown). These terminally differentiated HT29 cells finally died after 7–10 successive days, even when propagated in regular growth medium. Sodium-butyrate-supplemented medium had no effect on N59 glioblastoma and CCL185 NSCLC cells with regard to morphology (data not shown), [^3H]thymidine

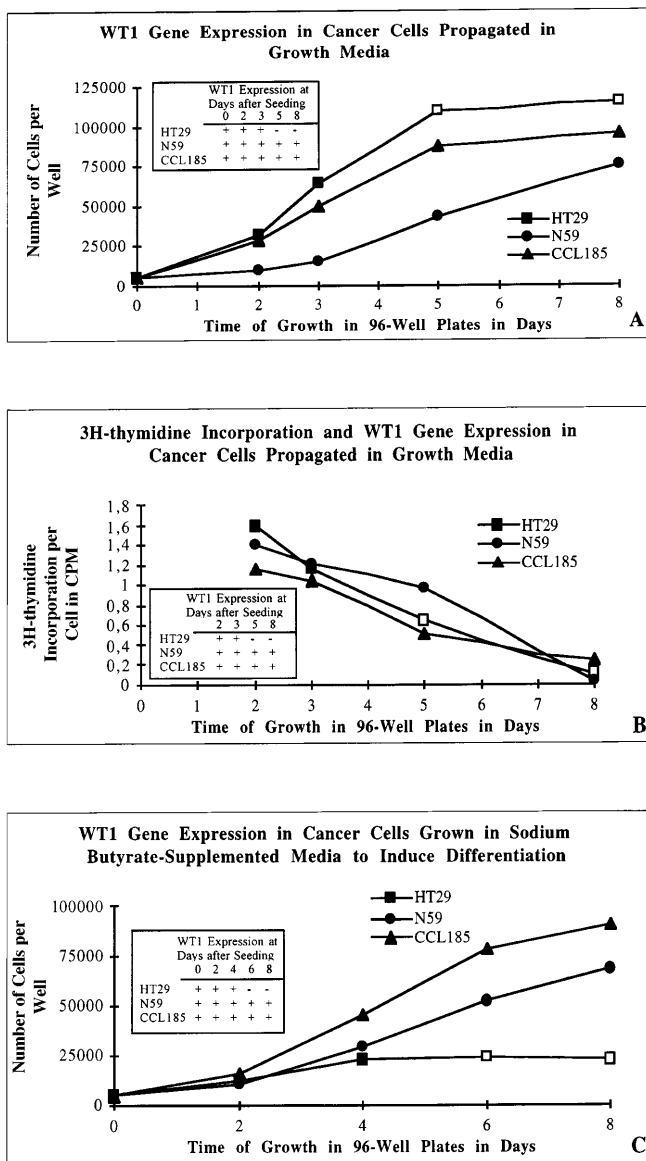


Fig. 2A–C *WT1* expression in HT29 colon cancer, N59 glioblastoma, and CCL185 NSCLC cells as it relates to growth and differentiation. **A** In HT29 cells, *WT1* expression is completely abrogated when cells reach confluence on days 5 and 8 (□, inset), but not when growing rapidly (■, ▲, ●). **A, B** N59 and CCL185 NSCLC cells continuously express *WT1* (■, ▲, ●, **A**), irrespective of growth and [³H]thymidine incorporation (**B**). **C** Unlike N59 and CCL185 NSCLC cells, differentiation-induced (sodium butyrate) HT29 cells stop growing and abrogate *WT1* expression (□, insets)

incorporation (data not shown), or growth rate (Fig. 2C). In contrast to HT29 cells, N59 and CCL185 cells continuously expressed *WT1* during in vitro growth (Fig. 2A–C).

Discussion

In conjunction with other transcription factors *WT1* expression regulates growth and differentiation of various tissue types. Like others (Brieger et al. 1994; Inoue

et al. 1996, 1994; Miwa et al. 1992; Miyagi et al. 1993), we (Menssen et al. 1997b, 1995) found *WT1* expression in blasts of the majority of acute leukemia patients, irrespective of lineage. Furthermore, it has been suggested for hemopoietic stem cells that a continuous over-expression of *WT1* acts leukemogenically (Inoue et al. 1997; Menssen et al. 1997a; Yamagami et al. 1996), since treatment of leukemic blasts with differentiation-inducing agents (Phelan et al. 1994; Sekiya et al. 1994) and *WT1* antisense oligonucleotides (Algar et al. 1996; Yamagami et al. 1996) down-regulates *WT1* expression and reduces cell growth. In the clinical setting, testing for *WT1* expression by RT-PCR is helpful to detect minimal residual disease and imminent relapses in acute leukemia patients (Brieger et al. 1995; Inoue et al. 1996; Menssen et al. 1998). Others have found *WT1* expression in the majority of human cancer cell lines of different origin, suggesting that it has a clinically important role in solid cancer, similar to its role in acute leukemia (Oji et al. 1999).

We therefore studied *WT1* expression in solid cancer cell lines and freshly isolated cancer tissue samples to elucidate whether data obtained from tumor cell lines can be transferred to the clinical setting. Because of their epidemiological relevance, we focused on lung and colon cancer patients.

The sensitivity and specificity of our *WT1* RT-PCR protocol has already been evaluated and validated elsewhere (Menssen et al. 1997b). This *WT1* RT-PCR protocol repeatedly detected 50 HL60 blasts intermingled with 10⁶ MNC from healthy volunteers. *WT1* expression was not found in MNC of healthy volunteers or in normal colon and lung tissue. By indirect immunofluorescence with mAb H2 (Rauscher et al. 1998), the *WT1* nuclear protein was detectable in HL60 blasts but in only a few cancer cell lines (NSCLC HTB57; glioblastoma cell lines N59, N64), confirming translation of *WT1*. From the combined evidence we conclude that our *WT1* RT-PCR protocol is suitable for detecting *WT1* expression.

We found *WT1* expression in most glioblastoma (63%) and many lung cancer (45%) and colorectal cancer (33%) cell lines. However, it was not detected in fresh colon cancer specimens, and only very few lung cancer (1 of 11) and glioblastoma (1 of 5) specimens expressed *WT1*. There are conflicting data on the frequency of *WT1* expression in human cancer cell lines. Some authors have found the gene to be expressed in 12 of 15 lung cancer (80%), 5 of 5 colon cancer (100%), 3 of 4 gastric cancer (75%), and 2 of 4 breast cancer cell lines (50%), using semi-quantitative RT-PCR (Oji et al. 1999), while others, using Northern blot analysis (Amin et al. 1995) or immunofluorescence (Kumar-Singh et al. 1997), found no *WT1* expression in lung cancer cell lines. However, the inferior sensitivity of Northern blot hybridization and indirect immunofluorescence compared to RT-PCR for detecting *WT1* expression can only partially explain this discrepancy, since Miyagi et al. found *WT1* expression in 36% of acute leukemia cell lines by

Northern blot analysis (Miyagi et al. 1993). Using RT-PCR, we detected *WT1* expression much less frequently in cancer cell lines than did Oje et al. In contrast to its use in acute leukemia therefore, testing for *WT1* expression can not be used to detect micrometastases or minimal residual disease in lung or colon cancer patients.

The high frequency of *WT1* expression in cancer cell lines compared to freshly isolated solid tumor samples may be caused by in vitro growth conditions. Similar results have been reported for *c-myc* gene expression. *c-myc* gene transcripts were detected in 40% of lung cancer cell lines but in only 10% of freshly isolated lung cancer tissue samples (Gazdar et al. 1985; Little et al. 1983). Furthermore, *WT1* was expressed in hematological malignancies by most undifferentiated, rapidly growing entities like acute lymphoblastic or myeloid leukemia, but not by slowly growing low-grade non-Hodgkin's lymphoma, such as chronic lymphocytic leukemia or follicular lymphoma (Brieger et al. 1994; Messen et al. 1995). The fastest growing and most undifferentiated tumor cell subpopulations are continuously selected because of the frequent splitting of in vitro cell cultures. This may explain the high frequency of *WT1* expression in some human cancer cell lines. We challenged this hypothesis by analyzing *WT1* expression in HT29 colon cancer cells at different phases of in vitro growth. There was a substantial reduction in HT29 cell growth upon reaching confluence 5 days after seeding. When the cells were grown to confluence, *WT1* expression could no longer be detected. However, HT29 cells expressed *WT1* during the logarithmic growth phase (days 1–4 after seeding). Moreover, induction of enteric differentiation in HT29 cells by sodium butyrate correlated to substantially reduced cell growth and loss of *WT1* expression. This phenomenon parallels K562 leukemic blasts, which down-regulate *WT1* expression when induced to erythrocytic differentiation (Phelan et al. 1994). In HT29 cells, sodium butyrate causes a cell-cycle G1-phase arrest. Ninety per cent of the HT29 cells were found in the G1/G0 phase of the cell cycle 24 h after sodium butyrate treatment (Barnard and Warwick 1993; Hodin et al. 1996). In conjunction with these results, our observations indicate an inverse correlation between *WT1* expression and cell-cycle G0/G1-phase arrest in HT29 cells. Conversely, this correlation supports the hypothesis that rapidly growing undifferentiated cancer cells are likely to express *WT1*.

We conclude that studies of *WT1* expression in lung cancer, colon cancer, and glioblastoma cell lines are useful for elucidating the biological function of *WT1* as it relates to cell-cycle progression and differentiation. However, in contrast to acute leukemia, testing for *WT1* expression has no clinical relevance for lung cancer, colon cancer, or glioblastoma patients.

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References

- Algar EM, Khromykh T, Smith SI, Blackburn DM, Bryson GJ, Smith PJ (1996) A *WT1* antisense oligonucleotide inhibits proliferation and induces apoptosis in myeloid leukaemia cell lines. *Oncogene* 12:1005–1014
- Amin KM, Litzky LA, Smythe WR, Mooney AM, Morris JM, Mews DJ, Pass HI, Kari C, Rodeck U, Rauscher FJ 3rd et al (1995) Wilms' tumor 1 susceptibility (*WT1*) gene products are selectively expressed in malignant mesothelioma. *Am J Pathol* 146:344–356
- Augeron C, Labois CL (1984) Emergence of permanently differentiated cell clones in a human colonic cancer cell line in culture after treatment with sodium butyrate. *Cancer Res* 44:3961–3969
- Barnard JA, Warwick G (1993) Butyrate rapidly induces growth inhibition and differentiation in HT-29 cells. *Cell Growth Differ* 4:495–501
- Bergmann L, Miething C, Maurer U, Brieger J, Karakas T, Weidmann E, Hoelzer D (1997) High levels of Wilms' tumor gene (*wt1*) mRNA in acute myeloid leukemias are associated with a worse long-term outcome. *Blood* 90:1217–1225
- Brieger J, Weidmann E, Fenchel K, Mitrou PS, Hoelzer D, Bergmann L (1994) The expression of the Wilms' tumor gene in acute myelocytic leukemias as a possible marker for leukemic blast cells. *Leukemia* 8:2138–2143
- Brieger J, Weidmann E, Maurer U, Hoelzer D, Mitrou PS, Bergmann L (1995) The Wilms' tumor gene is frequently expressed in acute myeloblastic leukemias and may provide a marker for residual blast cells detectable by PCR. *Ann Oncol* 6:811–816
- Bruening W, Gros P, Sato T, Stanimir J, Nakamura Y, Housman D, Pelletier J (1993) Analysis of the 11p13 Wilms' tumor suppressor gene (*WT1*) in ovarian tumors. *Cancer Invest* 11:393–399
- Call KM, Glaser T, Ito CY, Buckler AJ, Pelletier J, Haber DA, Rose EA, Kral A, Yeager H, Lewis WH, Jones C, Housmann DE (1990) Isolation and characterization of a zinc finger polypeptide gene at the human chromosome 11 Wilms' tumor locus. *Cell* 60:509–520
- Campbell CE, Kuriyan NP, Rackley RR, Caulfield MJ, Tubbs R, Finke J, Williams BR (1998) Constitutive expression of the Wilms' tumor suppressor gene (*WT1*) in renal cell carcinoma. *Int J Cancer* 78:182–188
- Gazdar AF, Carney DN, Nau MM, Minna JD (1985) Characterization of variant subclasses of cell lines derived from small cell lung cancer having distinct biochemical, morphological and growth properties. *Cancer Res* 45:2924–2930
- Gessler M, Poustka A, Cavenee W, Neve RL, Orkin SH, Bruns GAP (1990) Homozygous deletions in Wilms' tumours of a zinc-finger gene identified by chromosome jumping. *Nature* 343:774–778
- Hodin RA, Meng S, Archer S, Tang R (1996) Cellular growth state differentially regulates enterocyte gene expression in butyrate-treated HT-29 cells. *Cell growth and differentiation* 7:647–653
- Inoue K, Sugiyama H, Ogawa H, Nakagawa M, Yamagami T, Miwa H, Kita K, Hiraoka A, Masaoka T, Nasu K, et al (1994) *WT1* as a new prognostic factor and a new marker for the detection of minimal residual disease in acute leukemia. *Blood* 84:3071–3079
- Inoue K, Ogawa H, Yamagami T, Soma T, Tani Y, Tatekawa T, Oji Y, Tamaki H, Kyo T, Dohy H, Hiraoka A, Masaoka T, Kishimoto T, Sugiyama H (1996) Long-term follow-up of minimal residual disease in leukemia patients by monitoring

- WT1 (Wilms tumor gene) expression levels. *Blood* 88:2267–2278
- Inoue K, Ogawa H, Sonoda Y, Kimura T, Sakabe H, Oka Y, Miyake S, Tamaki H, Oji Y, Yamagami T, Tatekawa T, Soma T, Kishimoto T, Sugiyama H (1997) Aberrant overexpression of the Wilms tumor gene (WT1) in human leukemia. *Blood* 89:1405–1412
- Kumar-Singh S, Segers K, Rodeck U, Backhovens H, Bogers J, Weyler J, Van Broeckhoven C, Van Marck E (1997) WT1 mutation in malignant mesothelioma and WT1 immunoreactivity in relation to p53 and growth factor receptor expression, cell-type transition, and prognosis. *J Pathol* 181:67–74
- Ladanyi M, Gerald W (1994) Fusion of the EWS and WT1 genes in the desmoplastic small round cell tumor. *Cancer Res* 54:2837–2840
- Langerak AW, Williamson KA, Miyagawa K, Hagemeyer A, Versnel MA, Hastie ND (1995) Expression of the Wilms' tumor gene WT1 in human malignant mesothelioma cell lines and relationship to platelet-derived growth factor A and insulin-like growth factor 2 expression. *Genes Chromosomes Cancer* 12:87–96
- Little CD, Nau MM, Carney DN, Gazdar AF, Minna JD (1983) Amplification and expression of the c-myc oncogene in human lung cancer cell lines. *Nature* 306:194–196
- Maheswaran S, Park S, Bernard A, Morris JF, Rauscher FJ, Hill DE, Haber DA (1993) Physical and functional interaction between WT1 and p53 proteins. *Proc Natl Acad Sci USA* 90:5100–5104
- Menke AL, Shvarts A, Riteco N, Ham RCA van, Eb AJG van der (1997) Wilms' tumor 1-KTS isoforms induce p53-independent apoptosis that can be partially rescued by expression of the epidermal growth factor receptor or the insulin receptor. *Cancer Res* 57:1353–1363
- Menssen HD, Renkl HJ, Rodeck U, Maurer J, Notter M, Schwartz S, Reinhardt R, Thiel E (1995) Presence of Wilms' tumor gene (wt1) transcripts and the WT1 nuclear protein in the majority of human acute leukemias. *Leukemia* 9:1060–1067
- Menssen HD, Renkl H-J, Entezami M, Thiel E (1997a) Wilms' tumor gene expression in human CD34+ hematopoietic progenitors during fetal development and early growth (letter). *Blood* 89:3486–3487
- Menssen HD, Renkl HJ, Rodeck U, Kari C, Schwartz S, Thiel E (1997b) Detection by monoclonal antibodies of the Wilms' tumor (WT1) nuclear protein in patients with acute leukemia. *Int J Cancer* 70:518–523
- Menssen HD, Renkl HJ, Rieder H, Bartelt S, Schmidt A, Notter M, Thiel E (1998) Distinction of eosinophilic leukaemia from idiopathic hypereosinophilic syndrome by analysis of Wilms' tumor gene expression. *Br J Haematol* 101:325–334
- Miwa H, Beran M, Saunders GF (1992) Expression of the Wilms' tumor gene (WT1) in human leukemias. *Leukemia* 6:405–409
- Miyagi T, Ahuja H, Kubota T, Kubonishi I, Koeffler HP, Miyoshi I (1993) Expression of the candidate Wilms' tumor gene, WT1, in human leukemia cells. *Leukemia* 7:970–977
- Nagel WW, Vallee BL (1995) Cell cycle regulation of metallothionein in human colonic cancer cells. *Proc Natl Acad Sci* 92:579–583
- Oji Y, Ogawa H, Tamaki H, Oka Y, Tsuboi A, Kim EH, Soma T, Tatekawa T, Kawakami M, Asada M, Kishimoto T, Sugiyama H (1999) Expression of the Wilms' tumor gene WT1 in solid tumors and its involvement in tumor cell growth. *Jpn J Cancer Res* 90:194–204
- Park S, Schalling M, Bernard A, Maheswaran S, Shipley GC, Roberts D, Fletcher J, Shipman R, Rheinwald J, Demetri G, et al (1993) The Wilms tumor gene WT1 is expressed in murine mesoderm-derived tissues and mutated in a human mesothelioma. *Nat Genet* 4:415–420
- Phelan SA, Lindberg C, Call KM (1994) Wilms' tumor gene, WT1, mRNA is down-regulated during induction of erythroid and megakaryocytic differentiation of K562 cells. *Cell Growth Differ* 5:677–686
- Rauscher FJ 3rd, Morris JF, Fredericks WJ, Lopez-Guisa J, Balakrishnan C, Jost M, Herlyn M, Rodeck U (1998) Characterization of monoclonal antibodies directed to the amino-terminus of the WT1. Wilms' tumor suppressor protein. *Hybridoma* 17:191–198
- Rodeck U, Bossler A, Kari C, Humphreys CW, Gyorf T, Maurer J, Thiel E, Menssen HD (1994) Expression of the wt1 Wilms' tumor gene by normal and malignant human melanocytes. *Int J Cancer* 59:78–82
- Rose EA, Glaser T, Jones C, Smith CL, Lewis WH, Call KM, Minden M, Champagne E, Yeger H, Housemann DE (1990) Complete physical map of the WAGR region of 11p13 localizes a candidate Wilms' tumor gene. *Cell* 60:495–510
- Sekiya M, Adachi M, Hinoda Y, Imai K, Yachi A (1994) Down-regulation of Wilms' tumor gene (wt1) during myelomonocytic differentiation in HL60 cells. *Blood* 83:1876–1882
- Silberstein GB, Van Horn K, Strickland P, Roberts CT Jr, Daniel CW (1997) Altered expression of the WT1 wilms tumor suppressor gene in human breast cancer. *Proc Natl Acad Sci USA* 94:8132–8137
- Tanaka Y, Bush KK, Klauck KM, Higgins PJ (1989) Enhancement of Butyrate-induced differentiation of the HT-29 human carcinoma cells by 1,25-dihydroxyvitamin D3. *Biochem Pharmacol* 38:3859–3865
- Viel A, Giannini F, Capozzi E, Canzonieri V, Scarabelli C, Gloghini A, Boiocchi M (1994) Molecular mechanisms possibly affecting WT1 function in human ovarian tumors. *Int J Cancer* 57:515–521
- Walker C, Rutten F, Yuan X, Pass H, Mew DM, Everitt J (1994) Wilms' tumor suppressor gene expression in rat and human mesothelioma. *Cancer Res* 54:3101–3106
- Yamagami T, Sugiyama H, Inoue K, Ogawa H, Tatekawa T, Hirata M, Kudoh T, Akiyama T, Murakami A, Maekawa T (1996) Growth inhibition of human leukemic cells by WT1 (Wilms tumor gene) antisense oligodeoxynucleotides: implications for the involvement of WT1 in leukemogenesis. *Blood* 87:2878–2884