The role of Wilms' tumor genes

Masao Hirose

Division of Transfusion Medicine, The University of Tokushima School of Medicine, Tokushima, Japan

Abstract : The constitutional chromosomal deletion within the short arm of one copy of chromosome 11, at band p13, which often correlated with WAGR syndrome consisting of Wilms' tumor with aniridia, genitourinary malformation, and mental retardation, provided the first clue to the genetic events in the development of Wilms' tumor. *WT1* gene is encoded by 10 exons, resulting in messenger RNA subject to a complex pattern of alternative splicing. *WT1* gene encodes a zinc finger transcription factor, which binds to GC-rich sequences and functions as a transcriptional activator or repressor for many growth factor genes. WT 1 protein is mainly expressed in developing kidney, testis, and ovary, indicating that it is involved in the differentiation of genitourinary tissues, all thought to be the sites of origin of Wilms' tumor. The point mutation of *WT1* results in Denys-Drash syndrome. The other Wilms' tumor gene, *WT2* at 11p15.5, is linked to Beckwith-Wiedemann syndrome. The possibility that *WT1* is involved in the etiology of rhabdoid tumor of the kidney was discussed.

***WT1* is expressed in immortalized hematologic cells such as EBV-LCL and hematologic malignancies, but not in PBL or IL-2L. High level *WT1* expression in leukemia cells and a poor prognosis are linked in patients with leukemia, making the gene a novel marker for leukemia cells. A correlated expression between *WT1* and *mdr-1* in vincristine resistant cells indicates a close relation with multi-drug resistance and is a promising diagnositic marker for chemoresistance in hematologic malignancies. J. Med. Invest. 46 : 130-140, 1999**

Key words **:** *WT, tumor suppressor gene, Wilms' tumor, rhabdoid tumor, leukemia, chemoresistance*

Etiology of WAGR syndrome

In 1964, Miller *et al*. reported an association of Wilms 'tumor with aniridia, hemihypertrophy and other congenital malformations (1). A new disease entity, WAGR syndrome consisting of Wilms ' tumor with aniridia, genitourinary malformations, and mental retardation was subsequently proposed (2). Wilms 'tumor is the most common childhood intraabdominal solid tumor of the kidney (3). Thereafter, Riccardi *et al*. found a correlation between WAGR syndrome and karyotypic abnormality within the short arm of one copy of chromosome 11, at band p13 (4). This constitutional chromosomal deletion provided the first clue to the genetic events in the development of Wilms 'tumor.

In 1971, Knudson compared cases showing the earlier age of onset and the bilateral presentation of retinoblastomas (RB) in children with a family history of this disease with those showing the uni-

Abbreviations : PBL, peripheral blood lymphocytes ; IL-2L, interleukin 2 activated lymphocytes ; CML, chronic myelogenous leukemia ; EL, erythroleukemia ; MDS, myelodysplastic syndrome ; AML, acute myelogenous leukemia ; APL, acute promyelocytic leukemia ; AMoL, acute monocytic leukemia ; MegL, megakaryocytic leukemia ; EoL, eosinophilic leukemia ; cALL, common type acute lymphocytic leukemia ; T-ALL, T-cell type ALL ; ATL, adult T-cell leukemia/lymphoma ; T-CLL, T-chronic lymphocytic leukemia ; Afn BL, African Burkitt 's lymphoma ; B-ALL, B-cell type ALL ; Jpn BL, Japanese BL ; Imbl, immunoblastic type malignant lymphoma ; MM, multiple myeloma.

Received for publication May 25, 1999 ; accepted June 24, 1999.

Address correspondence and reprint requests to Masao Hirose, M.D., Division of Transfusion Medicine, The University of Tokushima School of Medicine, Kuramoto-cho, Tokushima 770-8503, Japan and Fax : +88-633-7211.

lateral tumors and proposed a model to explain the etiology of this disease, the two-event hypothesis (5). This model predicts that tumor formation depends on two-rate limiting genetic events. Tumorigenesis in children who have a constitutional lesion, either inherited from a parent or resulting from a spontaneous mutation, needed only one new genetic event. Contrary to this, sporadic cases required two independent somatic mutations. A similar model for Wilms 'tumor was proposed later (6). Subsequent genetic studies of a number of tumors confirmed that the two postulated genetic events are caused by the inactivation of both alleles of a tumor suppressor gene (7). Now, that Wilms 'tumor is caused by the Wilms 'tumor suppressor gene *WT1* is demonstrated. The Wilms 'tumor gene related diseases are summarized in Table 1.

Structure and regulatory mechanisms of WT1

The *WT1* gene that is mapped to chromosome locus 11p13 is encoded by 10 exons, resulting in messenger RNA subject to a complex pattern of alternative splicing (8, 9). The *WT1* promoter has three transcription initiation sites and the gene is a member of the GC-rich, TATA-less and CCAAT-less class of RNA polymerase II genes (10, 11). The *WT1* transcript is~3.5 kb long and encodes a zinc-finger protein, WT1, with a predicted molecular weight *Mr* of 47kd to 49kd, depending on the presence or absence of two alternatively spliced exons. The first alternative splicing introduces exon 5, encoding 17 amino acids (+17aa), just proximal to the first of four zinc fingers. The second results in an insertion of three amino acids (+KTS) between the third and fourth zinc fingers. The most prevalent *WT1* mRNA variant has both of the insertions present (+KTS and +17aa), and the least common form is missing both insertions (-KTS and -17aa) (9). The insertion of three amino acids (+KTS) disrupts the distance between zinc fingers 3 and 4 and thus alters its DNA-binding specificity (10). The four patterns of alternative splicing are shown in Figure 1.

The elements responsible for regulating the tissue specific expression of *WT1* are not known, although Sp1 and GATA-1 have been shown to positively modulate *WT1* expression (11-13). In addition, a cell type-specific enhancer has been identified within the 3 'end of the human *WT1* gene (14). Recently, Pax 2 and Pax 8 were shown to contribute to the regulation of *WT1*. When Pax 2 and Pax 8 expression becomes maximal, WT1 levels begin increasing (15). Recently, it was reported that the ectopic expression of p50 and p65 subunits of NF-κB stimulated the murine *WT1* promoter activity by 10-30-fold in a transient transfection assay (16). Subsequently, the regulators or signal cascades that could modulate the function of WT1 were studied. Evidence was obtained that WT1 protein expressed exogenously in fibroblasts was phosphorylated *in vivo* and that treatment with forskolin, which ac-

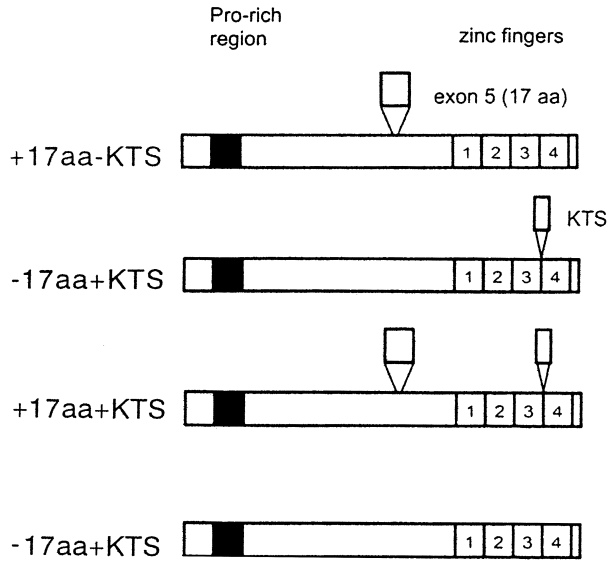

Figure 1. The four patterns of alternative splicing of the *WT1* gene. The details are explained in the text.

Table 1. Wilms 'tumor genes and related diseases

Disease name	Clinical manifestations	Genetic abnormality	Incidence of Wilms 'tumor
WAGR synd.	Aniridia, genitourinary malformations, mental retardation, Wilms 'tumor	Deletion at 11p13 (*WT1*)	> 30 %
Denys-Drash synd.	Intersexual disorders, nephropathy, Wilms 'tumor	*WT1* point mutation	> 90%
Beckwith-Wiedemann synd.	Macroglossia, organomegaly, hemihypertrophy, embryonal tumors	Duplication of paternal allele of 11p15 (*WT2*)	< 5 %

tivates the cAMP-dependent protein kinase (PKA) *in vivo*, induced phosphorylation of additional sites at Ser-365 and Ser-393 in WT1, resulting in abolishment of the DNA binding capacity of WT1 *in vitro* (17). In addition, Maheswaran and coworkers presented evidence that p53 physically interacts with WT1 in transfected cells and that WT1 transcriptional activity might be modulated by p53 (18).

Whereas in NIH-3T3 cells wild type WT1 repressed expression, its transfection into Saos-2 cells, which lack endogenous p53, resulted in increased transcription from a promoter into which epidermal growth factor receptor 1 (EGR-1) consensus binding sites had been added (18). However, the WT1 transcriptional activity was independent of *p53* genotype in the induction of P3 promoter activity of mouse insulin-like growth factor II gene (IGF-2) in primary cultures derived from p53 wild type (p53+/+) and knock-out (p53+/-) mouse embryo (19). There is a report that upregulation of *WT1* expression is associated with an expression marker for the differentiation into monocytes/macrophages of *c-fms* and *Mac-1* in murine myeloblastic cell line, M1, cells cultured in leukemia inhibitory factor (LIF), resulting in apoptotic cell death (20).

Functions of WT1

The *WT1* gene encodes a zinc finger transcription factor, which binds to GC-rich sequences (5'-GCGGGGGCG-3') and functions as a transcriptional activator or repressor (21). It represses transcription of growth factor [platelet derived growth factor-A (PDGF-A) chain, colony stimulating factor-1 (CSF-1), and IGF-2] and growth factor receptor (IGF-1R) genes, and the other genes [retinoic acid receptor-alpha (RAR-α), c-myc, and bcl-2](22-27). In addition, it's been known that *WT1* mediates the expression of genes such as EGR-1, EGF-R, inhibin-α, Pax2, transforming growth factor-beta (TGF-β) and *WT1* itself (28-33). The transient transfection of *WT1* constructs into NIH-3T3 or 293 cells results in the transcriptional repression of a number of co-transfected promoters containing the EGR-1 consensus sequence (34). The EGR-1 consensus is found upstream of many transcriptional start sites, leading to the identification of a number of promoters that bind *in vitro*-translated *WT1* and are repressed in transient transfection assay. Haber and coworkers transfected the four wild type *WT1* isoforms into RM1 human anaplasia of the kidney cell line, a variant form of Wilms' tumor, inoculated into nude mice and observed that each isoform independently suppressed the emergence of colony formation (35). Subsequently, they found that stable transfection of *WT1* into RM1 cells results in induction of endogenous IGF-2 but not of other previously postulated WT1 target genes (34).

WT1 is expressed only in specific types of cells, which is a major difference from *RB* or *p53* (36). The pattern of normal *WT1* expression has provided important clues to the function of *WT1* during differentiation. The WT1 protein is mainly expressed in developing kidney, testis, and ovary, indicating that it is involved in the differentiation of genitourinary tissues. Other organs expressing the protein are spleen and the methothelial cells of heart, lung and abdomen (36-38). In the kidney, *WT1* is expressed only in condensing blastemic cells, renal vesicles, and glomerular epithelium, all thought to be sites of origin of Wilms' tumor. Renal *WT1* expression peaks around the time of birth and then rapidly declines as the organ matures (37, 38). In contrast to its transient expression in the developing kidney, *WT1* is expressed continuously in methothelial cells, sertoli cells of testis, and granulosa cells of the ovary (39). The critical developmental role of *WT1* is evident in the severe genitourinary and methothelial abnormalities of mice whose *WT1* alleles have been deleted. *WT1* knock-out mice die before birth with failure of kidney and gonadal development in addition to hypoplasia of the heart and lungs (40).

The WT1 protein is present in normal breast tissue and appears to be developmentally regulated in that a high percentage of breast tumor cells express little or no WT1 protein. It is known that breast tumor growth is controlled by the genes encoding components of the IGF and TGF-β signaling system and, recently, altered expression of *WT1* in breast cancer was also reported (41).

WT2 at 11p15

The second Wilms' tumor gene was identified at chromosome locus 11p15 and designated *WT2* (42, 43). This locus is linked to the Beckwith-Wiedemann syndrome (BWS), showing manifestations such as visceromegary, macroglossia, or hemihypertrophy in some patients (43, 44). *p57* was recently identified as a cyclin-dependent kinase (CDK) inhibitor,

which is found to have the strongest tumor suppressor activity among the genes of the *p21* family (45, 46). As shown in Figure 2, like related family members such as *p21* and *p27*, *p57* binds to several G_1-cyclin/CDK complexes and arrests the cell cycle at the G_1 phase (47). Human *p57* is found at chromosome locus 11p15.5, a region implicated in the etiology of embryonal tumors including Wilms' tumor and BWS. Frequent loss of 11p15.5 in Wilms' tumor indicates that *WT2* is located in this region (45, 48).

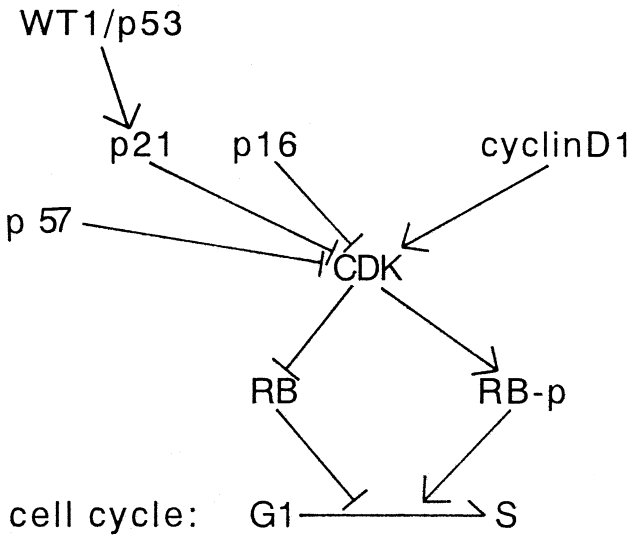

Figure 2. The relation between the proteins that regulate cell cycle progression.

Role in rhabdoid tumor of the kidney

The author established two cell lines of rhabdoid tumor of the kidney (RTK), SWT-1 and SWT-2, as shown in Figure 3 (49). RTK was once proposed to be a variant of Wilms' tumor and subsequently segregated from an unfavorable type of sarcomatous renal tumor that was grouped with clear cell sarcoma (CCSK) and anaplasia of the kidney (50-53). It is well known that RTK has a much poorer prognosis than Wilms' tumor (50, 52, 53). In an attempt to identify the etiology of RTK, the mRNA expression of genes including *WT1*, *p57*, *IGF-2*, *p53*, *N-myc*, and *c-myc* was tested using reverse transcriptase-polymerase chain reaction (RT-PCR) in the two cell lines. The expressions were compared with those in a biopsied kidney tissue and a normal mesangeal cell line, MCP-3. Coinciding with the karyotype of SWT-2 cell lines, del 11p13 as shown in Figure 4, no mRNA expression of

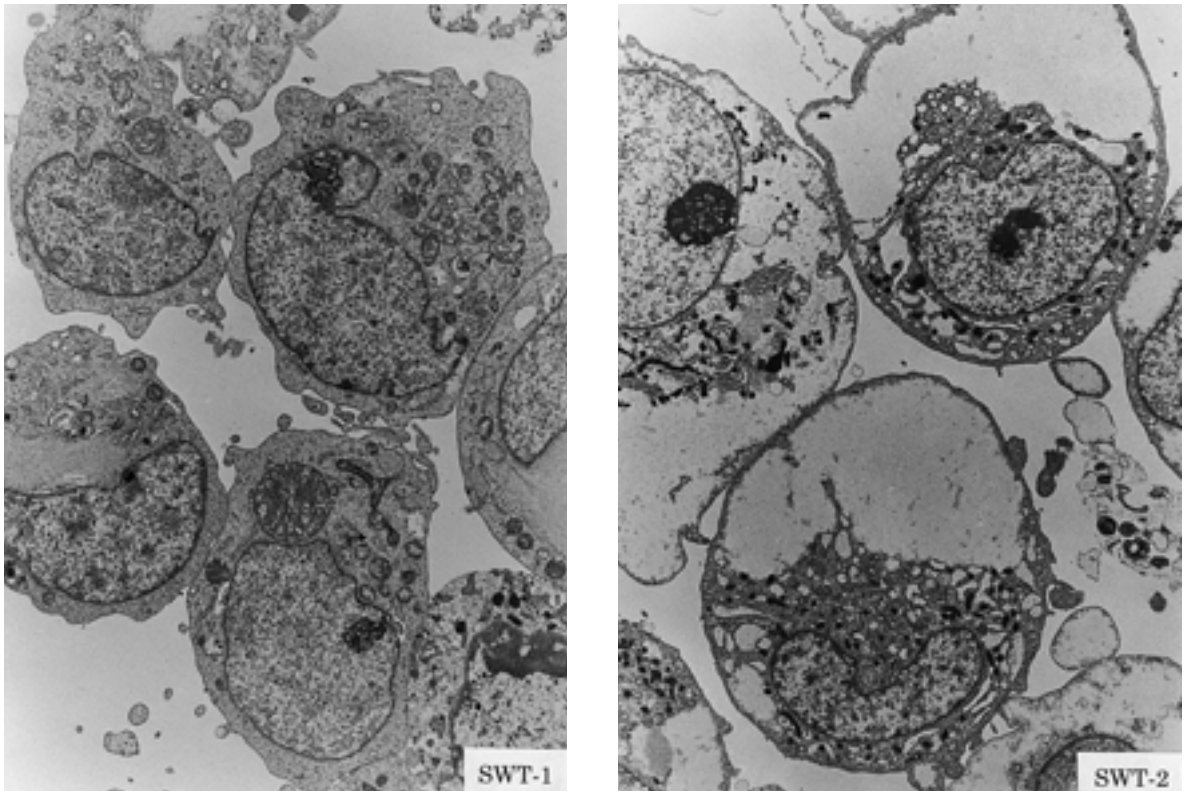

Figure 3. Ultrastructure of SWT-1 and SWT-2. Intermediate filaments characteristic for the rhabdoid tumor can be seen around the perinuclear field.

WT1 was found. Interestingly, SWT-1, which showed a normal karyotype at least on the G-banding analysis, also lacked the *WT1* expression as shown in Figure 5. In addition, SWT-2 lacked the expression of two genes, *p57* and *IGF-2*, while SWT-1 expressed them. Both *N-myc* and *c-myc* expression was detected in the two cell lines (data not shown). These results strongly suggest that *WT1* is involved in the etiology of RTK. However, transient transfection of *WT1* did not reduce colony formation in the other RTK cell line, SM 2 (35). The etiology of RTK may need other genetic abnormalities in addition to *WT1*. The presence of *p57* mRNA in SWT-1 implies that *WT2* is not involved in the etiology of this disease.

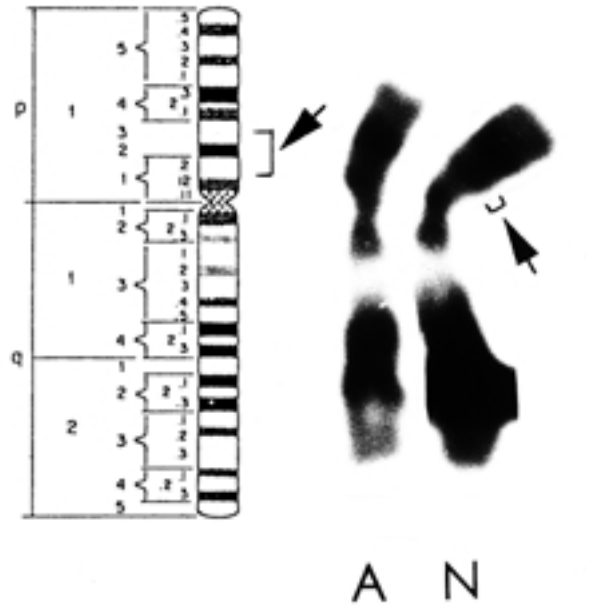

Figure 4. Structure of the short arm of chromosome 11 and the karyotype of SWT-2. SWT-2 shows del 11p13. The *WT1* and aniridia gene of *Pax6* are located at band 11p13, and *p57* and *IGF-2* are located at band 11p15.5.

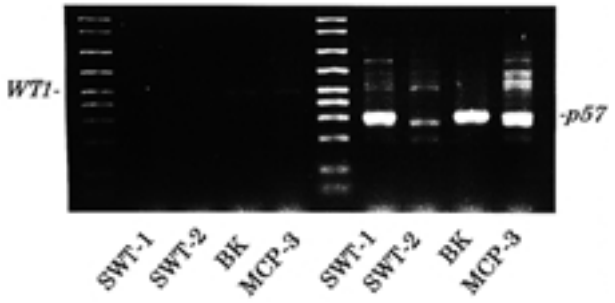

Figure 5. No *WT1* mRNA expression in two RTK cell lines. Neither RTK cell line expresses *WT1* whereas the biopsied kidney sample (BK) and normal mesangeal cell line (MCP-3) show weakly positive expression.

Other functions of WT1

cDNA constructs encoding the four human *WT1* splice variants caused programmed cell death, which was associated with a reduced synthesis of EGF-R in human osteosarcoma cell lines, U2OS and Saos-2 (54). The effect of this assay was independent of the presence of the *p53* gene. However, the nature of the recipient cells themselves is an important factor in these transient transfection studies. WT1 and p53 proteins are associated *in vivo* and transfection of *WT1* into cells deleted of the *p53* gene results in transcriptional activation, an effect that is suppressed by the reinduction of wild-type p53 (18). *WT1* was transiently transfected into the p53-negative Hep3B and the p53-positive Hep2G hepatoma cell lines, resulting in the induction of apoptosis in both cell lines by the wild type splice variant, whereas the *WT1* (+KTS) isoform did not induce apoptosis (55). This result is independent of the effect of p53, which seems to contradict a previous publication in which the expression of the *WT1* (-/-) isoform was reported to suppress p53-induced apoptosis in both U2OS and E1A-transformed baby rat kidney (18). Thus, the observation that the effect of the *WT1* isoforms is cell type dependent may explain these discrepancies. Subsequently, it was demonstrated that inducible expression of *WT1* in osteosarcoma cells triggers programmed cell death through an effect that is associated with transcriptional repression of endogenous EGFR. This *WT1* mediated apoptosis was preceded by induction of the CDK inhibitor p21, associated with G1 phase arrest (56).

Microinjection of the *WT1* cDNA into quiescent cells or cells in early to mid G1 phase blocked serum-induced cell cycle progression into S phase, from which inhibition of the activity of cyclin/CDK complexes may be involved in mediating the *WT1*-induced cell cycle block (57).

Mutation of WT1

Mutations in the *WT1* gene underlie 5 % to 10 % of cases of sporadic Wilms 'tumor (58). Athough the majority follow Knudson 's two-hit hypothesis for tumor suppressor genes, it is now clear that a substantial minority (~30 %) of Wilms 'tumors retain one normal *WT1* allele, suggesting that in some cases, heterozygous mutation is sufficient for tumorigenesis. Five different types of mutations

are commonly found in Wilms 'tumors : large deletions of part of the gene, nonsense or frameshift mutations affecting amino acids in the zinc fingers critical for DNA binding, missense mutations affecting the putative activation or repression domains, and mutations preventing correct soliciting. Approximately 75 % of the *WT1* mutations found in sporadic Wilms 'tumor produce a truncated protein, whereas missense mutations in the zinc finger region predominate in Denys-Drash syndrome (DDS)(58). Wilms 'tumor patients have an increased frequency of leukemias as second primary tumors, some of which may be due to *WT1* mutation (59). The biological significance of DNA binding and transcriptional regulation by WT1 is underscored by the finding of small deletions and point mutations in the *WT1* zinc-fingers that abolish DNA binding in a number of Wilms 'tumors, especially in tumors associated with the DDS (60). Consequently, the ability of the mutant *WT1* allele, containing an in-frame deletion within the DNA-binding domain, to transform baby rat kidney was tested. The mutant *WT1* gene was found to cooperate with the adenoviral *E1A* gene in transforming baby rat kidney cells, whereas the wild-type *WT1* gene in all of its alternatively spliced forms neither suppressed E1A-induced focus formation in soft agar nor cooperated with E1A (61).

Expression in hemopoetic cells

The *WT1* gene is strongly over-expressed in leukemic blasts compared with immature hematopoetic progenitors with an increase in its expression levels at relapse and an inverse correlation between its expression levels and prognosis, making it a novel marker for leukemia cells. The poor prognosis and the higher level of *WT1* gene expression are linked in patients with leukemia. Thus, it was supposed that the aberrant over-expression of *WT1* contributed to the pathogenesis of AML (62, 63). In addition, *WT1* was shown to be useful in the monitoring of minimal residual disease in patients with hematologic malignancy. However, a consequential result that normal CD34 positive stem cells as well as acute myeloid leukemia cells equally express *WT1* mRNA was obtained (64).

It was reported that K562 cells down regulate *WT1* mRNA during induced erythroid and megakaryocytic differentiation (65). The same phenomenon was observed in HL60 cells, suggesting that sustained high levels of *WT1* are incompatible with differentiation (66). Likewise, the expression of *WT1* inversely correlated with the differentiation level of acute leukemias (67). *WT1* antisense oligomers inhibit the cell growth of both leukemic cell lines and fresh leukemic blasts from patients with acute leukemia or chronic myeloid leukemia, indicating that the WT1 protein may be important for the sustained proliferation of leukemia cells (68). Contrary to these findings, the monoblastic cell line, U937, constitutively expressing either of the isoforms, (-KTS) or (+KTS), did not respond to differentiation induction by retinoic acid or vitamin D3, whereas the cell line unable to express this gene responded to these substances (69). A recent result indicated that *WT1* expression competes with the differentiation-inducing signal mediated by G-CSF receptor and constitutively activated Stat3, resulting in the blocking of differentiation and subsequent proliferation (70). *WT1* mutation is associated with a failure to achieve complete remission and a lower survival rate in AML, confirming that *WT1* mutations underlie a similar proportion of cases of AML to that seen in Wilms ' tumors and its normal role in hemopoesis is at a very early progenitor stage (71).

Relation between WT1 and multidrug resistance (MDR)

The drug resistance that is a major obstacle to cancer treatment develops at the initial stage of the therapy or during chemotherapy after relapse (72). p170-kd permeability-related glycoprotein (P-GP) encoded by the *mdr-1* gene has ATPase activity which reduces the intracellular drug accumulation by increasing the efflux of anti-cancer drugs from cells (73, 74). p190-kd multidrug resistance-associated protein (MRP) encoded by the *mrp* gene is a member of the ATP-binding cassette super family of membrane transporter proteins that displays minor homology to P-GP (75). It is known that both P-GP and MRP are expressed in normal peripheral blood lymphocytes (PBL), monocytes, and granulocytes as well as cells of hematologic disease. However, the spectrum of cells expressing P-GP is narrow as compared with those expressing MRP, which exists in a wider spectrum of cells or tissues than P-GP (76-79). In recent years, a 110-kd lung resistance-related protein (LRP) has been found

(80), which is reported to be expressed in monocytes among the bone marrow cells (81).

In an attempt to understand the cause of the poor prognosis of leukemia patients whose leukemia cells expressed a high level of *WT1*, the author investigated the relation between *WT1* and multidrug resistance (MDR). The expression level of the *WT1* gene in human hemopoetic cells such as peripheral blood lymphocytes (PBL), interleukin-2 activated lymphocytes (IL-2L), the lymphoblastoid cell lines immortalized by EBV (EBV-LCL), and the cultured cell lines derived from human hematologic malignancies was compared with that of *mdr-1*, *mrp*, and *lrp* in Table 2. Positive expressions of MDR-related genes as above were detected in all PBL, IL-2L, and EBV-LCL, while the *WT-1* expression was found in immortalized cell lines such as EBV-LCL and hematologic malignancies. However, the results using normal cells or wild type cell lines did not demonstrate any positive correlation between MDR-related genes and *WT1*. Subsequently, the author tested the expression of *WT1* in vincristine (VCR) resistant cells. As shown in Figure 6, in three VCR-resistant cells that induced *mdr-1*, *WT1* correlated with the progression of drug resistance and the increase of *mdr-1* expression. The disappearance of *p53* may suggest a linkage with *mdr-1* and *WT1*. Thus, it became obvious that the high level of *WT1* expression is a promising diagnostic marker for drug resistance (82). Another study reported the relation between *WT1* mutations and chemoresistance (81). Although it is apparent that the *WT1* gene is linked to the poor prognosis that is caused by the chemoresistance in hematologic malignancies, little is known of the role of *WT1*. The role of *WT1* in the hematologic malignancies merits further investigation.

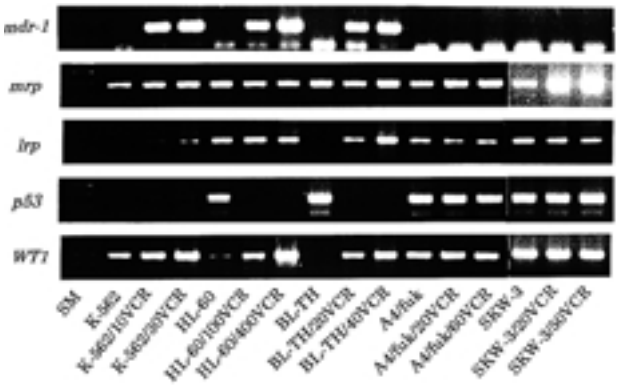

Figure 6. The mRNA expression of *WT1* and MDR-related genes in vincristine resistant hematologic cell lines. The PCR was initiated with 50 ng of cDNA and every PCR went through 28 cycles because the products increased linearly until 30 PCR cycles. Products were analyzed using NIH image.

Table 2. Expression of MDR-related genes and WT1

Lineage	Cell lines	Origin	mdr-1	mrp	lrp	WT-1
PBL	PBL	PBL	2+	2+	1+	-
			2+	2+	2+	-
			2+	2+	2+	-
			2+	2+	2+	-
T-cell	IL-2L	PBL	2+	2+	2+	-
			2+	2+	2+	-
			2+	2+	2+	-
			+	2+	2+	-
			2+	2+	1+	-
B-cell	EBV-LCL	PBL	w	2+	2+	+
			+	2+	1+	+
			+	w	1+	+
			+	2+	2+	w
			2+	2+	2+	2+
			w	w	2+	+
			+	+	2+	+
Myeloid	K-562	CML	+	+	w	+
	KOPM-28	CML	-	2+	-	-
	HEL	EL	+	2+	1+	w
	P39/tsu	MDS	-	+	2+	2+
	ML-1	AML	-	w	2+	2+
	PL-21	APL	-	2+	2+	+
	HL-60	APL	-	+	2+	+
	THP-1	AMoL	-	+	2+	2+
	P31/fuj	AMoL	-	2+	2+	2+
	CMK	MegL	+	+	+	+
	EoLE5	EoL	-	w	1+	2+
nT, nB	P30/ohk	cALL	-	+	2+	+
	Reh	cALL	-	2+	2+	-
	KOPN-1	cALL	-	2+	2+	+
	KM-3	cALL	-	2+	2+	+
	Nalm-6	cALL	-	2+	2+	2+
T-cell	P12/ich	T-ALL	-	2+	1+	2+
	CCRF-CEM	T-ALL	-	2+	2+	+
	RPMI-8402	T-ALL	-	+	w	2+
	MOLT-3	T-ALL	-	2+	2+	2+
	MOLT-4F	T-ALL	-	+	1+	+
	HPB-ALL	T-ALL	-	2+	w	2+
	ATL-1K	ATL	-	2+	1+	-
	MT-2	ATL	+	w	2+	-
	SKW-3	T-CLL	-	2+	2+	2+
B-cell	Raji	Afn, BL	-	w	-	w
	P3HR-1	Afn, BL	-	w	-	w
	Daudi	Afn, BL	-	+	-	2+
	BALL-1	B-ALL	-	2+	-	-
	BL-TH	Jpn, BL	-	2+	-	-
	P32/ish	Jpn, BL	-	2+	-	-
	A3/kaw	Imbl	+	2+	-	-
	A4/fuk	Imbl	-	2+	1+	2+
	U-266	MM	-	+	2+	w

The level of expression was analyzed using NIH image and the positive expression was divided into three levels by a proper criterion : -, negative expression ; positive expression was described in the order of w<1+<2+.

REFERENCES

1. Miller RW, Fraumeni JFJr, Manning MD: Association of Wilms 'tumor with aniridia, hemihypertrophy and other congenital malformations. N Engl J Med 270 : 922-927, 1964
2. Turleau C, Grouchy J, Dufier JL, Phuc LH, Svhmelck PH, Rappaport R, Nihoul-Fekete C, Diebold N : Aniridia, male pseudohermaphroditism, gonadoblastoma, mental retardation, and del 11p13. Hum Genet 57 : 300-306, 1981
3. National Wilms 'Tumor Study Committee: Wilms 'tumor : status report. 1990. J Clin Oncol 9 : 877-887, 1991
4. Riccardi VM, Sujansky E, Smith AC, Franke U : Chromosomal imbalance in the aniridia-Wilms ' tumor association : 11p interstitial deletion. Pediatrics 61 : 604-610, 1978
5. Knudson AGJr : Mutation and cancer ; statistical study of retinoblastoma. Proc Natl Acad Sci USA 68 : 820-823, 1971
6. Knudson AGJr, Strong LC : Mutation and cancer ; a model for Wilms 'tumor of the kidney. J Natl Cancer Inst 48 : 313-324, 1972
7. Comings DE : A general theory of carcinogenesis. Proc Natl Acad Sci USA 70 : 3324-3328, 1973
8. Call M, Glaser H, Ito CY, Buckler AJ, Pelletier J, Haber DA, Rose EA, Kral A, Yeger H, Lewis WH, Jones C, Housman DE : Isolation and characterization of a zinc-finger polypeptide gene at the human chromosome 11 Wilms ' tumor locus. Cell 60 : 509-520, 1990
9. Haber DA, Sohn RL, Buckler AJ, Pelletier J, Call KM, Housman DE : Alternative splicing and genomic structure of the Wilms 'tumor gene WT1. Proc Natl Acad Sci USA 88 : 9618-9622, 1991
10. Pelletier J, Schalling M, Buckler AJ, Rogers A, Haber DA, Housman DE : Expression of the Wilms 'tumor gene WT1 in the murine urogenital system. Genes Dev 5 : 1345-1356, 1991
11. Hofmann W, Royer HD, Dresler M, Schneider S, Royer-Pokora B : Characterization of the transcriptional regulatory region of the human WT1 gene. Oncogene 8 : 3123-3132, 1993
12. Cohen HT, Bossone SA, Zhu G, McDonald GA, Sukhatme VP : Sp1 is a critical regulator of the Wilms 'tumor-1 gene. J Biol Chem 272 : 2901-2913, 1997
13. Wu Y-J, Fraizer GC, Saunders GF : GATA-1 transactivates the WT1 hematopoietic specific enhancer. J Biol Chem 270 : 5944-5949, 1995
14. Fraizer GC, Wu Y-J, Hewitt SM, Maity T, Ton CCT, Huff V, Saunders GF : Transcriptional regulation of the human Wilms 'tumor gene (WT1). J Biol Chem 269 : 576-588, 1994
15. Dehbi M, Pelletier J : Pax 8-mediated activation of the WT1 tumor suppressor gene. EMBO J 15 : 4297-4306, 1996
16. Dehbi M, Hiscott J, Pelletier J : Activation of the WT1 Wilms 'tumor suppressor gene by NF-κB. Oncogene 16 : 2033-2039, 1998
17. Sakamoto Y, Yoshida M, Semba K, Hunter T : Inhibition of the DNA-binding and transcriptional repression activity of the Wilms 'tumor gene product, WT1, by cAMP-dependent protein kinase-mediated phosphorylation of Ser-365 and Ser-393 in the zinc finger domain. Oncogene 15 : 2001-2012, 1997
18. Maheswaran S, Park S, Bernard A, Morris JF, Rauscher FJ III, Hill DE, Haber DA : Physical and functional interactions between WT1 and p53 proteins. Proc Natl Acad Sci USA 90: 5100-5104, 1993
19. Duarte A, Caricasole A, Graham CF, Ward A : Wilms 'tumor-suppressor protein isoforms have opposite effects of IGF2 expression in primary embryonic cells, independently of p53 genotype. Brit J Cancer 77 : 253-259, 1998
20. Smith SI, Weil D, Johnson GR, Boyd AW, Li CL : Expression of the Wilms 'tumor suppressor gene, WT1, is upregulated by leukemia inhibitory factor and induces monocytic differentiation in M1 leukemic cells. Blood 91: 764-773, 1998
21. Wang Z-Y, Qui Q-Q, Gurrieri M, Huang J, Deuel TF : Products of alternatively spliced transcripts of the Wilms 'tumor suppressor gene, wt1, have altered DNA binding specificity and regulate transcription in different ways. Oncogene 10 : 415-422, 1995
22. Haber DA, Buckler AJ, Glaser T, Call KM, Pelletier J, Sohn RL, Douglass EC, Housman DE : An internal deletion within an 11p13 zinc finger gene contributes to the development of Wilms 'tumor. Cell 61 : 1257-1269, 1990
23. Gashler AL, Bonthron DT, Madden SL, Rauscher FJ III, Collins T, Sukhatme VP : Human platelet-derived growth factor A chain is transcriptionally repressed by the Wilms 'tumor suppressor WT1. Proc Natl Acad Sci USA 89 : 10894-10988, 1992
24. Harrington MA, Konicek B, Song A, Xia X-L,

Fredericks WJ, Rauscher FJ III : Inhibition of colony-stimulating growth factor-1 promoter activity by the product of the Wilms 'tumor locus. J Biol Chem 268 : 21271-21275, 1993
25. Drummond IA, Madden SL, Rohwer-Nutter P, Bell GI, Sukhatme VP, Rauscher FJ III : Repression of the insulin-like growth factor II gene by the Wilms 'tumor suppressor WT1. Science 257 : 674-678, 1992
26. Werner H, Re GG, Drummond IA, Sukhatme VP, Rauscher FJ III, Sens DA, Garvin AJ, LeRoith D, Roberts CT Jr : Increased expression of the inslin-like growth factor 1 receptor gene, IGF1R, in Wilms 'tumor is correlated with modulation of IGF1R promoter activity by the WT1 Wilms 'tumor gene product. Proc Natl Acad Sci USA 90 : 5828-5832, 1993
27. Goodyer P, Dehbi M, Torban E, Bruening W, Pelletier J : Repression of the retinoic acid receptor-alpha gene by the Wilms 'tumor suppressor gene product, wt1. Oncogene 10 : 1125-1129, 1995
28. Hewitt SM, Hamada S, McDonnel TJ, Rauscher FJ III, Saunders GF : Regulation of the proto-oncogenes bcl-2 and c-myc by the Wilms 'tumor suppressor gene WT1. Cancer Res 55 : 5386-5389, 1995
29. Madden SL, Cook DM, Morris JF, Gashler A, Sukhatme VP, Rauscher FJ III : Transcriptional repression mediated by the WT1 Wilms 'tumor gene product. Science 253 : 1550-1553, 1991
30. Englert C, Hou X, Maheswaran S, Bennett P, Ngwu C, Re GG, Garvin AJ, Rosner MR, Haber DA : WT1 suppresses synthesis of the epidermal growth factor receptor and induces apoptosis. EMBO J 14 : 4662-4675, 1995
31. Hsu SY, Kubo M, Chun SY, Halsuka FG, Housman DE, Hsueh AJ : Wilms 'tumor protein WT1 as an ovarian transcription factor : decreases in expression during follicle development and repression of inhibin-alpha gene promoter. Mol Endocrinol 9 : 1356-1366, 1995
32. Ryan G, Steele-Perkins V, Morris JF, Rauscher FJ III, Dressler GR : Repression of Pax-2 by WT1 during normal kidney development. Development 121 : 867-875, 1995
33. Dey BR, Sukhatme VP, Roberts AB, Sporn MB, Rauscher FJ III, Kim SJ : Repression of the transforming growth factor-beta gene by the Wilms 'tumor suppressor WT1 gene product. Mol Endocrinol 8 : 595-602, 1994
34. Nicolas KE, Re GG, Yan YX, Garvin AJ, Haber DA : WT1 induces expression of insulin-like growth factor 2 in Wilms 'tumor cells. Cancer Res 55 : 4540-4543, 1995
35. Haber DA, Park S, Maheswaran S, Englert C, Re GG, Hazen-Martin DJ, Sens DA, Garvin AJ : WT1-mediated growth suppression of Wilms 'tumor cells expressing a WT1 splicing variant. Science 262 : 2057-2059, 1993
36. Rupprecht HD, Drummond IA, Madden SL, Rauscher FJ III, Sukhatme VP : The Wilms 'tumor suppressor gene WT1 is negatively autoregulated. J Biol Chem 269 : 6198-6206, 1994
37. Prichard-Jones K, Fleming S, Davidson D, Bickmore W, Porteous D, Gasden C, Bard J, Buckler A, Pelletier J, Housman D : The candidate Wilms 'tumor gene is involved in genitourinary development. Nature 346 : 194-197, 1990
38. Buckler AJ, Pelletier J, Haber DA, Glaser T, Housman DE : Isolation, characterization, and expression of the murine Wilms 'tumor gene (WT1) during kidney development. Mol Cell Biol 11 : 1707-1712, 1991
39. Park S, Schalling M, Bernard A, Maheswaran S, Shipley GC, Roberts D, Fletcher J, Shipman R, Rheinwald J, Demetri G : The Wilms 'tumor gene WT1 is expressed in murine mesoderm-derived tissues and mutated in a human methothelioma. Nat Genet 4 : 415-420, 1993
40. Kreidberg JA, Sariola H, Loring JM, Maeda M, Pelletier J, Housman D, Jaenish R : WT1 is required for early kidney development. Cell 74 : 679-691, 1993
41. Silverstein GB, Horn KV, Strickland P, Roberts CTJr, Daniel CW : Altered expression of the WT1 Wilms tumor suppressor gene in human breast cancer. Proc Natl Acad Sci USA 94: 8132-8137, 1997
42. Mannens M, Slater RM, Heyting C, Bliek J, Kraker J, Coad N, de Pagter-Holthuizen P, Pearson PL : Molecular nature of genetic changes in loss of heterozygosity of chromosome 11 in Wilms 'tumors. Hum Genet 81 : 41-48, 1988
43. Koufos A, Grundy P, Morgan K, Alerk KA, Hadro T, Lampkin BC, Kalbakji A, Cavenee WK : Familial Wiedemann-Beckwith syndrome and a second Wilms 'tumor locus both map to 11p15.5 Am J Hum Genet 44 : 711-719, 1989
44. Ping AJ, Reeve AE, Law DJ, Young MR, Boehnke M, Feinberg AP : Genetic linkage of Beckwith-Wiedemann syndrome to 11p15. Am J Hum Genet 44 : 720-723, 1989
45. Matsuoka S, Edwards MC, Bai C, Parker S,

Zhang P, Baldini A, Harper JW, Elledge SJ : p57 KIP2, a structurally distinct member of the p21 CIP1 Cdk inhibitor family, is a candidate tumor suppressor gene. Genes & Dev 9 : 650-662, 1995
46. Loe DW, Deeley RG, Cole SPC : Biology of the multidrug resistance-associated protein, MRP. Europ. J. Cancer 32A : 945-957, 1996
47. Hunter T, Pines J : Cyclins and cancer. II: cyclin D and CDK inhibitors come of age. Cell 79 : 573-582, 1994
48. Junien C, Henry I : Genetics of Wilms 'tumor : A blend of aberant and genomic imprinting. Kidney Int 46 : 1264-1279, 1994
49. Hirose M, Yamada T, Abe T, Hirose T, Shimizu E, Yamamoto Y, Kagami S, Takano S, Yamaguchi T, Kuroda Y : Establishment and characterization of two cultured cell lines derived from malignant rhabdoid tumors of the kidney. Int J cancer 67 : 218-223, 1996
50. Beckwith JB, Palmer NF : Histological and prognosis of Wilms 'tumor : Results from the First National Wilms 'Tumor Study. Cancer 41 : 1937-1948, 1978
51. Morgan E, Kidd JM : Undifferentiated sarcoma of the kidney : A tumor of childhood with histopathologic and clinical characteristics distinct from Wilms 'tumor. Cancer 42 : 1916-1921, 1978
52. Haas JE, Palmer NF, Weinberg AG, Beckwith JB : Ultrastructure of malignant rhabdoid tumor of the kidney : A distinctive renal tumor of children. Hum Pathol 12 : 646-657, 1981
53. Weeks DA, Beckwith JB, Mierau GW, Luckey DW : Rhabdoid tumor of kidney. A report of 111 cases from the National Wilms 'Tumor study pathology center. Am J Surg Pathol 13 : 438-458, 1989
54. Englert C, Hou X, Maheswaran S, Bennett P, Ngwu C, Re GG, Garvin AJ, Rosner MR, Haber DA : WT1 suppresses synthesis of the epidermal growth factor receptor and induces apoptosis. EMBO J 14 : 4662-4675, 1995
55. Menke AL, Shvarts A, Riteco N, van Ham RCA, van der Eb AJ, Jochemsen AG : 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, 1997
56. Englert C, Maheswaran S, Garvin AJ, Kreidberg J, Haber DA : Induction of p21 by the Wilms ' tumor suppressor gene WT1. Cancer Res 57 : 1429-1434, 1997
57. Kudoh T, Ishidate T, Moriyama M, Toyoshima K, Akiyama T : G1 phase arrest induced by Wilms 'tumor protein WT1 is abrogated by cyclin/CDK complexes. Proc Natl Acad Sci USA 92 : 4517-4521, 1995
58. Little M, Wells C : A clinical overview of WT1 gene mutations. Hum Mutat 9 : 209-225, 1997
59. Moss TJ, Strauss LC, Das L, Feig SA : Secondary leukemia following successful treatment of Wilms 'tumor. Am J Pediatr Hematol Oncol 11 : 158-161, 1989
60. Wang Z-Y, Qiu Q-Q, Engler KT, Deuel TF : A second transcriptionally active DNA-binding site for the Wilms 'tumor gene product, WT1. Proc Natl Acad Sci USA 90 : 8896-8900, 1993
61. Haber DA, Timmers HTM, Pelletier J, Sharp PA : A dominant mutation in the tumor gene WT1 cooperates with the viral oncogene E1A in transformation of primary kidney cells. Proc Natl Acad Sci USA 89 : 6010-6014, 1992
62. Miwa H, Beran M, Saunders GF : Expression of the Wilms 'tumor gene (wt1) in human leukemia. Blood 90 : 1217-1225, 1997
63. Inoue K, Sugiyama H, Ogawa H, Nakagawa M, Yamagami T, Miwa H, Kita K, Hiraoka A, Masaoka T, Nasu K, Kyo T, Dohy H, Nakauchi H, Ishidate T, Akiyama T, Kishimoto T : WT1 as a new prognostic factor and a new marker for the detection of minimal residual disease in acute leukemia. Blood 84 : 3071-3079, 1994
64. Mauer U, Weidemann E, Karakas T, Hoelzer Z, Bergmann L : Wilms tumor gene (wt1) mRNA is equally expressed in blast cells from acute myeloid leukemia and normal CD 34+ progenitors. Blood 90 : 4230-4232, 1997
65. Phelan SA, Lindberg C, Call KM : Wilms 'tumor gene, WT1, mRNA is down regulated during induction of erythroid and megakaryocytic differentiation of K562 cells. Cell Growth Differ 5 : 677-686, 1994
66. Sekiya M, Adachi M, Hinoda Y, Imai K, Yachi A : Down regulation of Wilms 'tumor gene (WT1) during monocytic differentiation in HL60 cells. Blood 83 : 1876-1882. 1994
67. Patmasiriwat P, Fraizer GC, Claxton D, Kantarjian H. Saunders GF : Expression pattern of WT1 and GATA-1 in AML with chromosome 16q22 abnormalities. Leukemia 10 : 1127-1133, 1996
68. Algar EM, Khromykh T, Smith SI, Blakburn DM, Bryson GJ, Smith PJ : A WT1 antisense

oligonucleotide inhibits proliferation and induces apoptosis in myeloid leukemia cell line. Oncogene 12 : 1005-1014, 1996
69. Svedberg H, Chylicki K, Baldetorp B, Rauscher FJ III, Gullberg U : Constitutive expression of the Wilms 'tumor gene (WT1) in the leukemic cell line U937 blocks parts of the differentiation program. Oncogene 16 : 925-932, 1998
70. Inoue K, Tamaki H, Ogawa H, Oka Y, Soma T, Tatekawa T, Oji Y, Tsuboi A, Kim EH, Kawakami M, Akiyama T, Kishimoto T, Sugiyama H: Wilms 'tumor gene (WT1) competes with differentiation-inducing signal in hematopoietic progenitor cells. Blood 91 : 2969-2976, 1998
71. King-Underwood L, Prichard-Jones K : Wilms ' tumor (WT1) gene mutations occur mainly in acute myeloid leukemia and may confer drug resistance. Blood 91 : 2961-2968, 1998
72. Thottassery JV, Zambetti GP, Arimori K, Schuetz EG, Schetz JD : p53-dependent regulation of mdr1 gene expression causes selective resistance to chemotherapeutic egents. Proc Natl Acad Sci USA 94 : 11037-11042 1997
73. Pastan I, Gottesman M : Multiple drug resistance in human cancers. New Engl J Med 316 : 1288-1293, 1987
74. Tsuruo T : Mechanisms of multidrug resistance and implications for therapy. Jpn J Cancer Res 79 : 285-296, 1988
75. Cole SPC, Bhardwaj G, Gerlach JH, Mackie JE, Grant CE, Almquist KC, Stewart AJ, Kurz EU, Duncan AMV, Deeley RG : Overexpression of a transporter gene in a multidrug-resistant human lung cancer cell line. Science 258: 1650-1654, 1992
76. Dimiani D, Michieli M, Michelutti A, Geromin A, Raspsdori D, Fanin R, Savignano C, Giacca M, Pileri S, Mallardi F, Baccarani M : Expression of multidrug resistance gene (MDR-1) in human normal leukocytes. Hematologica 78 : 12-17, 1993
77. Kang YK, Zhan Z, Regis J, Robey R, Meadows B, Dickstein B, Lee JS, Otsuki T, Stetler-Stevenson M, Jaffe ES, Solomon D, Wilson WH, Fojo A, Bates S : Expression of mdr-1 in refractory lymphoma : quantitation by polymerase chain reaction and validation of the assay. Blood 15 : 1515-1524, 1995
78. Kavallaris M, Leary JA, Barrett JA, Friedlander ML : MDR1 and multidrug-associated protein (MRP) gene expression in epithelial ovarian tumors. Cancer Lett 102 : 7-16, 1996
79. Esteller M, Martines-Palones JM, Garcia A, Xercavins J, Reventos J : High rate of mdr-1 and heterogenous pattern of mrp expression without gene amplification in endometrial cancer. Int J Cancer 69 : 798-803, 1995
80. Scheffer GL, Wijingaard OLJ, Flens MJ, Izquierdo MA, Slovak ML, Pinedo HM, Meijer CJLM, Clevers HC, Scheper RJ : The drug resistance related protein LRP is the human major vault protein. Nat Med 1 : 578-582, 1995
81. Sugawara I, Akiyama S, Scheper RJ, Itoyama S : Lung resistance protein (LRP) expression in human normal tissues in comparison with that of MDR 1 and MRP. Cancer Lett 112 : 23-31, 1997
82. Hirose M, Kuroda Y : p53 may mediate mdr-1 expression via the WT1 gene in human vincristine-resistant leukemia/lymphoma cell lines. Cancer Lett 129 : 165-171, 1998