

Invited Review

The *yin-yang* of PR-domain family genes in tumorigenesis

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Summary. Cancer is essentially caused by alterations in normal cellular genes. Multiple gene changes involving at least two types of cancer genes, protooncogenes and tumor suppressor genes, are required for the clonal expansion of a malignant cell. This discussion focuses on the recently recognized role of a small but expanding family of PR-domain genes in tumorigenesis. The protein products of these genes are involved in human cancers in an unusual *yin-yang* fashion. Two products are normally produced from a PR-domain family member which differ by the presence or absence of the PR domain; the PR-plus product is disrupted or underexpressed whereas the PR-minus product is present or overexpressed in cancer cells. This imbalance in the amount of the two products, a result of either genetic or epigenetic events, appears to be an important cause of malignancy.

Key words: PR-domain, *RIZ*, *MDSI-EVII*, *Blimp1*, Tumor suppressors, SET-domain

Cloning of *RIZ* gene and identification of the PR domain

One of the best studied tumor suppressor genes is the retinoblastoma susceptibility gene *Rb*. *Rb* is believed to act as a cell cycle break to stop cell growth when the need arises, such as when cells are committed to undergo differentiation. *Rb* acts by protein complex formation with DNA binding proteins to modulate nuclear DNA related events. This activity of *Rb* is regulated by G1-specific cyclin-dependent kinases. The essential role of *Rb* in tumorigenesis is underscored by the observation that nearly all of the components of the *Rb* pathway are altered in tumor cells (Weinberg, 1996). The *Rb*-interacting zinc finger gene *RIZ* was isolated in a functional screening for *Rb*-binding proteins (Buyse et

al., 1995) and independently as a GATA-3-binding protein G3B (Shapiro et al., 1995) and as a DNA-binding protein *MTB-Zf* (Muraosa et al., 1996). Although the role of *RIZ* in *Rb* function remains to be determined, *RIZ* itself has proven to be a very interesting gene consistent with its potential in the *Rb* pathway.

One immediate novel feature recognized from the full-length *RIZ* cDNA sequences of rat and human is the PR domain (Fig. 1). This domain represents an ~100-amino acid region of homology first found between *RIZ* and the previously cloned *PRDI-BFI* protein (*PRDI-BFI-RIZI* homologous region) (Huang, 1994; Buyse et al., 1995). The murine homolog of *PRDI-BFI* was later independently cloned as *Blimp1* (Keller and Maniatis, 1991; Turner et al., 1994).

When the *RIZ1* PR domain peptide sequence was later used as a query to search the translated nucleotide database of Genbank (by the *tblastn* program), we found that a portion of the 5' untranslated region of the *EVII* oncogene encodes the B and C boxes of the PR domain, and the *MDSI* gene 5' to the *EVII* locus encodes the A box of PR (Fears et al., 1996). Indeed, the expression of the fusion *MDSI-EVII* gene has been experimentally confirmed (Fears et al., 1996). Furthermore, the *Caenorhabditis elegans* homolog of *MDSI-EVII* gene *egl-43*, which controls motor neuron migration, also has a PR domain (Garriga et al., 1993). Thus, the *MDSI-EVII* gene is a PR gene that normally produces at least two different length products, the PR-containing *MDSI-EVII* protein and the PR-lacking *EVII* protein.

The recently completed *C. elegans* genome sequence revealed two more PR domain-containing open reading frames. One of these F25D7.3 is the homolog of *BLIMP1* because the zinc finger domains are also highly homologous. The other T21B10.5 is devoid of zinc finger domains and is the first example of a PR protein without any zinc finger domains. EST clones of PR domain genes are also found in the *Drosophila* EST databases. However, no PR peptides can be detected in the yeast genome. Thus, the PR domain may have evolved as a result of the special need of multicellular organisms.

The PR domain is primarily associated with the

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Table 1. Subclassification of C₂H₂ zinc finger genes.

CLASSES	NUMBERS			FUNCTIONS	MUTATED IN CANCER
	Human	<i>C. elegans</i>	<i>S. cerevisiae</i>		
PR ^a	3~12	2	0	dimerization	<i>MDS1-EV11</i>
POZ ^a	25~75	2	0	dimerization	<i>Bcl6, PLZF</i>
KRAB	70-245	0	0	repressor	
FAX ^b	0	0	0	?	
Other ^c	c 110-385	153	45		<i>WT1</i>
Total	300-700	157	45		
% genes ^d ~0.7%	~0.7%	~0.7%	~0.7%		

^a: The number of genes in each domain class reflects only the number of C₂H₂ zinc finger genes that have such a domain. Both the number of known genes and the estimated total number are given. Thus, 3 PR genes are known and a total of 12 is expected in the human genome. The PR domain and POZ domain are also present in non-zinc finger proteins. A total of 3 PR proteins and 157 POZ proteins are found in *C. elegans* (Chervitz et al., 1998; Clarke and Berg, 1998). The PR domain is not found in any yeast proteins although its related SET domain is. The POZ is found in 4 yeast proteins.

^b: The FAX domain is only found in frogs. ^c: Genes of this group have no commonly shared N-terminal modules. ^d: The number of C₂H₂ zinc finger genes divided by the total number or estimated number of genes in each species (Chervitz et al., 1998; Clarke and Berg, 1998).

C₂H₂ or Krüppel-like family zinc finger genes. The C₂H₂ zinc finger family is estimated to consist of 300-700 human genes (Bellefroid et al., 1989). This family can be further divided into different subsets based on other structural features, which include several conserved amino-terminal modules such as KRAB, POZ (also BTB and ZIN), and FAX domains (Knochel et al., 1989; Bellefroid et al., 1991; Rosati et al., 1991; Numoto et al., 1993; Zollman et al., 1994). The PR domain represents a newly recognized such module. A comparison of these modules is shown in Table 1. The subfamily represented by PR is the smallest. Unlike its larger sister families whose members may have diverse biological functions, PR family members share common biological functions in tumorigenesis as discussed below.

PR domain function and relationship with the SET domain

Except for the single *C. elegans* gene *T21B10.5*, all other known PR genes contain multiple classical C₂H₂ zinc finger domains and likely function as DNA binding proteins. All three human PR genes have been shown to bind to specific DNA sequences and to either activate or repress transcription. The PR domain of RIZ1 lacks either an activator or repressor function, indicating that a direct role in transcriptional regulation may not be a conserved function of the PR domain (Xie et al., 1997).

A common function of the PR domain is likely to be mediating protein-protein interaction. The PR domain of RIZ1 functions as a protein-binding interface (Huang et al., 1998). Recombinant PR domain protein can bind to in vitro translated RIZ1 protein in vitro. Binding is mediated by residues conserved among different PR domains, suggesting that similar functions may be shared among different PR domains.

Using a recently developed, more powerful database-searching program (PSI-BLAST), the PR

domain was found to be homologous to the previously recognized SET domain (Huang et al., 1998). The SET domain is a 130-amino acid, evolutionarily conserved sequence motif present in chromosomal proteins that function in modulating gene activities from yeast to mammals (Tschiersch et al., 1994; Stassen et al., 1995). It is important to note that the shared residues between PR and SET are also among the most conserved residues in each domain, suggesting they may share a common function (Fig. 2). Indeed, several different SET domains have recently been shown to mediate protein-protein interactions (Cardoso et al., 1998; Cui et al., 1998; Rozenblatt-Rosen et al., 1998). Thus, PR and SET are related protein-protein interaction modules.

Relative to PR genes, more have been learned about SET genes. These genes have diverse biological

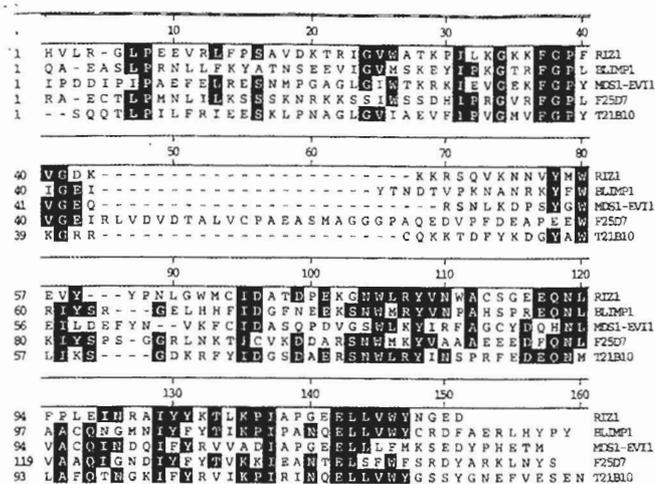


Fig. 1. PR domain alignment. The PR domain sequences of three human genes and two *C. elegans* open reading frames are aligned. Residues that are conserved in at least three proteins are shaded in black.

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functions related to chromatin structure. They play an important role in development, cancer, position-effect-variegation (PEV), telomeric and centromeric gene silencing, and possibly in determining chromosome architecture (for a review, see Jenuwein et al., 1998). They are multifunctional chromatin regulators with activities in both eu- and heterochromatin. The founding members of the family include three *Drosophila* genes: the PEV suppressor gene *Su(Var)3-9* (Tschiersch et al., 1994), the polycomb group gene *Enhancer of zeste* (Jones and Gelbart, 1993), and the trithorax group gene *trithorax* (Kennison, 1995). Other members include the *S. cerevisiae* *SET1* gene, which affects mating-type switching and telomeric silencing (Nislow et al., 1997), the *S. pombe* *Clr4+* gene, which is involved in centromere function (Ekwall et al., 1996), and the human *trithorax* homolog *HRX* (also called *ALL-1* and *MLL*), which is a breakpoint gene involved in human leukemia (Djabali et al., 1992; Gu et al., 1992; Tkachuk et al., 1992).

Despite the sequence similarity between PR and SET, which is typically ~20% identical in amino acids, they are clearly distinctive because identities among PR genes are typically ~45% and among SET genes ~50%. Also SET domains are primarily found at the carboxyl-termini of proteins, whereas PR domains are mostly located at the amino-termini. PR domains are primarily associated with zinc finger proteins whereas SET domains have been found in a variety of proteins containing various motifs including the chromo domain,

A/T hooks, zinc finger, PHD fingers, and GTP-binding motifs (Jenuwein et al., 1998). Finally, SET is found in yeast genome, suggesting that PR is likely a derivative of SET domain in evolution. This relationship with SET suggests that PR domain genes are members of a superfamily that function to assemble chromatin-based multiprotein complexes involved in either euchromatin-mediated gene activation or heterochromatin-mediated gene silencing.

Expression of alternative PR-minus products

An interesting and unusual feature of some PR genes is the generation of an alternative product that lacks PR but is otherwise identical to the PR-plus product (Fig. 3). This was first found for the *MDS1-EV11* gene as mentioned above. The EV11 product lacks the PR domain whereas MDS1-EV11 has. Both products are expressed in normal tissues. *EV11* is generated by an internal promoter located within the *MDS1-EV11* gene (Bartholomew and Ihle, 1991). The promoter for the full length *MDS1-EV11* gene has yet to be isolated. The MDS1 exon is located >170 kb 5' of the EV11 promoter, indicating that the *MDS1-EV11* gene is very large (Fears et al., 1996). In addition, *egl-43*, the *C. elegans* homolog of *MDS1-EV11*, produces an alternative product lacking the PR domain (Fig. 3), suggesting that expression of the

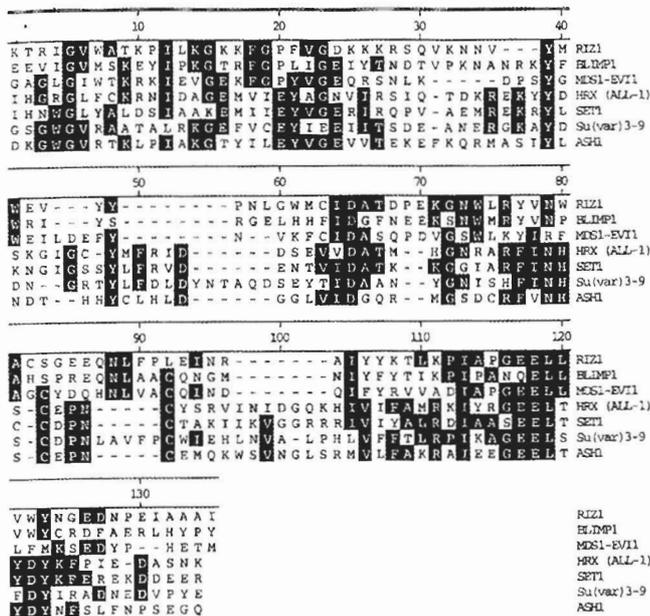


Fig. 2. Alignment of PR domains and SET domains. The PR domain region of RIZ1, MDS1-EV11 and BLIMP1 proteins are aligned with the SET domain regions of ALL-1 (human), SET1 (yeast), Su(var)3-9 (*Drosophila*) and ASH1 (*Drosophila*) proteins. Residues that are identical in at least three proteins are shaded.

PR (*PRDI-BF1* and *RIZ* homology) domain family

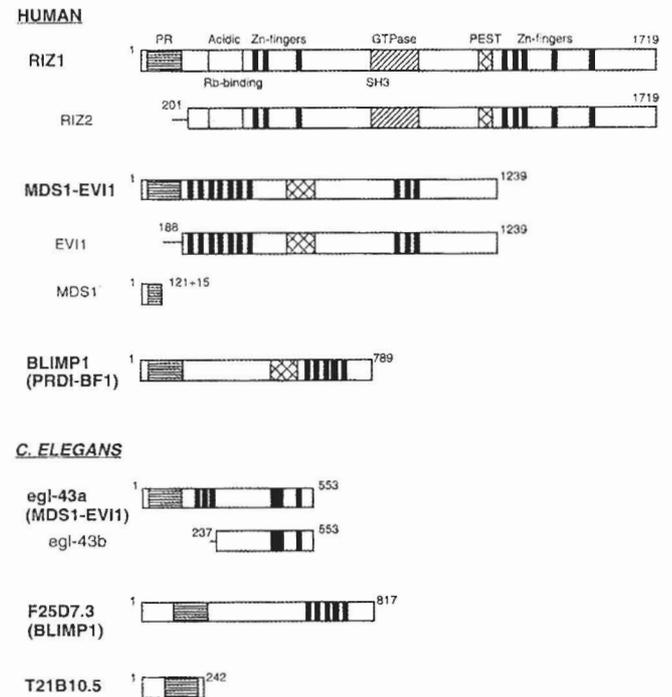


Fig. 3. PR domain family members. Schematics of protein products of three human PR genes and three *C. elegans* genes are shown. *MDS1-EV11* and *BLIMP1* genes have *C. elegans* homologs.

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PR-minus product by a PR gene is evolutionarily conserved.

The *RIZ* gene also produces a PR-minus product. When the protein products of *RIZ* were first identified in tumor cell lines, only a single protein species of 250 kDa was recognized (Buyse et al., 1995). This protein was later found to be lacking the PR domain and was designated RIZ2 protein. The full-length PR-containing product RIZ1 was identified as a 280-kDa protein that is at a much lower level than RIZ2 in all tumor cell lines examined (Liu et al., 1997). Characterization of the human *RIZ* gene genomic structure established that RIZ2 is produced by an internal promoter (Liu et al., 1997). *RIZ* gene is very large (>150 kb) and consists of at least 10 exons. A complete genomic sequence for the 3' end of *RIZ* is available in Genbank (AL031277), which is ~150 kb. This sequence contains a single CpG island, indicative of promoters. Reassuringly, the RIZ2 promoter we identified previously is located within this

CpG island. The PR domain is encoded by three small exons 3-5 (the first coding exon is designated exon 1). The majority of the *RIZ1* cDNA is encoded by one large exon, coding exon 7. RIZ2 mRNA is produced by an internal promoter located at the intron-exon boundary of coding exon 5. The RIZ2 promoter from human, rat, and mouse genes have been isolated and sequenced. The promoter is highly conserved and shows features of a typical TATA-less GC-rich promoter of a housekeeping gene, consistent with the ubiquitous expression in all normal and tumor tissues examined (Liu et al., 1997).

Whether the *BLIMP1* gene may express a PR-lacking product remains to be investigated. While internal promoter is used in the cases of *MDS1-EV11* and *RIZ*, other mechanisms such as alternative splicing is theoretically possible in generating PR-minus products.

The yin-yang of PR genes in tumorigenesis

Many different lines of investigation suggest that PR genes are involved in cell growth control and tumorigenesis. They do so in an unusual yin-yang fashion. In particular, the PR-containing products play a role in tumor suppression while the PR-minus products are oncogenic or at least play a different role from the PR-plus products. Inactivation of PR-plus products or activation of PR-minus products or both are commonly observed in cancer cells.

Disruption of *MDS1-EV11* and activation of *EV11* in myeloid leukemia

A large body of evidence show that the *MDS1-EV11* locus is genetically involved in human and murine myeloid leukemia. As mentioned above, *MDS1-EV11* was originally identified as two separate genes, *MDS1* and *EV11*, on chromosome 3q26. *MDS1* was cloned as one of the partner genes of *AML1* in the t(3;21)(q26;q22), associated with therapy-related acute myeloid leukemia and myelodysplastic syndrome as well as with chronic myeloid leukemia in blast crisis (Nucifora et al., 1994). The protooncogene *EV11* was first identified in the mouse and is activated in murine myeloid leukemia by proviral insertion in the *EV11* common integration site (Morishita et al., 1988). Both *MDS1-EV11* and *EV11* are not abundantly expressed in normal hematopoietic cells. In humans, *EV11* can be activated in myeloid leukemias and myelodysplastic diseases by chromosomal rearrangements at either 5' or 3' of the gene (Morishita et al., 1992a,b; Mitani et al., 1994; Nucifora et al., 1994). Activation of *EV11* also occurs as part of the fusion mRNA, *AML1-EV11* or *AML1-MDS1-EV11* (Figs. 4, 5). There is also evidence of *EV11* overexpression in leukemia and solid tumors in the absence of any detectable chromosomal abnormalities (Russell et al., 1994; Brooks et al., 1996), suggesting a more frequent role for *EV11* in tumorigenesis involving epigenetic mechanisms. *EV11* is capable of transforming rodent fibroblast cells (Kurokawa et al., 1995). It

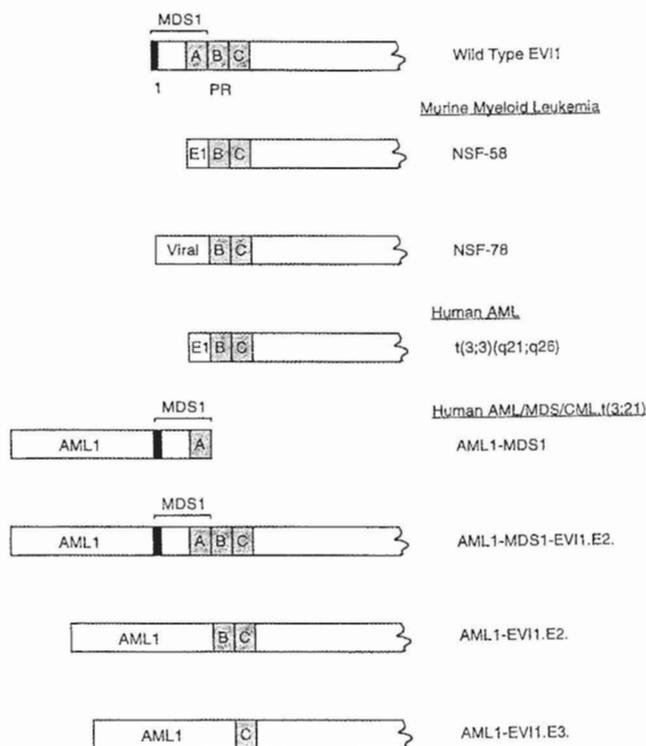


Fig. 4. Summary of altered forms of *MDS1-EV11* gene expressed in mouse and human leukemia. A schematic of the partial wild-type *MDS1-EV11* protein structure is shown. The PR domain is shown as shaded boxes with the three exons labeled as A, B and C. Viral insertions in murine myeloid leukemia result in the activation of the *EV11* promoter producing the *EV11* transcript in NSF-58 cell line. In the NSF-78 cell line, viral sequences actually fuse with *EV11*. In human AML, the t(3;3)(q21;q26) translocation activates the transcription of *EV11*. In human AML/MDS/CML involving t(3;21), various fusion transcripts are detected, including AML1-MDS1, AML1-MDS1-EV11.E2 (MDS1 is fused with the exon 2 of *EV11*), AML1-EV11.E2 and AML1-EV11.E3. In none of these cases, a wild-type *MDS1-EV11* protein is expressed as a result of chromosome alterations.

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interferes with hematopoietic cell differentiation (Morishita et al., 1992a,b; Kreider et al., 1993). These observations are consistent with *EVII*'s role as an oncogene.

In contrast to *EVII*, *MDS1-EVII* is rarely activated or overexpressed in tumor cells, suggesting that the function of this gene may be incompatible with, if not suppressing, tumor cell growth (Fig. 5). In fact, chromosomal translocations or viral insertions consistently disrupt the PR domain of *MDS1-EVII* without affecting the structure of the PR-minus product *EVII* (Fig. 4). As a result, the *EVII* gene becomes overexpressed and is thought to directly contribute to malignant transformation. *MDS1-EVII* overexpression has occasionally been observed in the form of a fusion gene with *AML1* (Fig. 4). However, such a fusion product is likely to be functionally different from the wild type *MDS1-EVII*.

The new realization of the disruption of *MDS1-EVII* raises questions as to whether inactivation of *MDS1-EVII* may also contribute to transformation, in addition to the oncogenic action of *EVII*. Chromosomal translocation that disrupts *MDS1-EVII*, followed by loss of the remaining wild-type allele (or loss of heterozygosity LOH), would result in a complete lack of *MDS1-EVII* function in a tumor cell. Even in the absence of LOH, it is possible that a reduced dosage of *MDS1-EVII*, due to disruption of one copy, could be tumorigenic. Also, the overexpressed *EVII* may exert a dominant negative effect on the *MDS1-EVII* protein function or at least alter the normal yin-yang balance of the two proteins. Genetically engineered animal models where *MDS1-EVII* is disrupted but *EVII* is normal should be

generated to test whether loss of *MDS1-EVII* alone is sufficient for tumor formation.

Common loss of *RIZ1* but never *RIZ2* expression in human cancers

Several observations suggest that *RIZ1* is a tumor suppressor. *RIZ1* maps to the distal short arm of human chromosome 1 next to the marker D1S228 on 1p36.23 (Buyse et al., 1996; Muraosa et al., 1996), which commonly undergoes deletions, rearrangements, or LOH in a broad spectrum of human tumors, including mammary cancer (Genuardi et al., 1989), ovarian cancer (Thompson et al., 1997), primary hepatoma (Simon et al., 1991), colorectal cancer (Bardi et al., 1993), chronic myelocytic leukemia (Mori et al., 1998), non-Hodgkin's lymphoma (Mitelman et al., 1997), melanoma (Dracopoli et al., 1989), parathyroid adenoma (Williamson et al., 1997), Merkel cell carcinoma (Harnett et al., 1991), pheochromocytoma (Vargas et al., 1997), and neuroblastoma (Fong et al., 1992). *RIZ1* gene expression is commonly lost in human breast cancer cell lines and tumor specimens as well as in other types of tumors (He et al., 1998). In contrast, *RIZ2* is uniformly expressed in all cases examined. The uniform presence of *RIZ2* suggests that loss of *RIZ1* is not a random-occurring event. There may be a specific negative selection for *RIZ1* versus *RIZ2* in tumors. Consistently, forced *RIZ1* expression causes G2/M cell cycle arrest and/or apoptosis (He et al., 1998).

Inactivating gene expression rather than intragenic mutations affecting protein structure appears to be the basis of *RIZ1* alteration in malignant cells. Whether tumor-associated 1p36 alterations may inactivate *RIZ1* expression requires future investigation. Relative to the *RIZ1* abnormality, the uniform presence of *RIZ2* is striking and may indicate a positive role for *RIZ2* in oncogenesis. A need to maintain *RIZ2* expression in tumor cells may explain the lack of gross mutations in *RIZ* because *RIZ2* shares 89% of the coding region with *RIZ1*. Of course, mutations in the PR region of *RIZ1* should not affect *RIZ2*. Such mutations, however, must be subtle (undetectable by Southern-blot analysis). Moreover, if such mutations exist, they are likely to be rare because tumors primarily display *RIZ1* under-expression. However, it is possible that certain nucleotide changes could lead to destabilization of transcripts. Given that *RIZ1* and *RIZ2* are produced by different promoters, it seems likely that the *RIZ1* promoter may represent a specific target of inactivation in tumor cells.

Although loss of *RIZ1* expression may be common in human cancers, the present lack of data of intragenic mutations in *RIZ1* makes it difficult to distinguish causality from correlation. To prove that loss of *RIZ1* is causal to human tumorigenesis, animal models will be needed where *RIZ1* but not *RIZ2* is specifically inactivated or knocked out. We have in fact generated such models in the mouse and found those animals to be

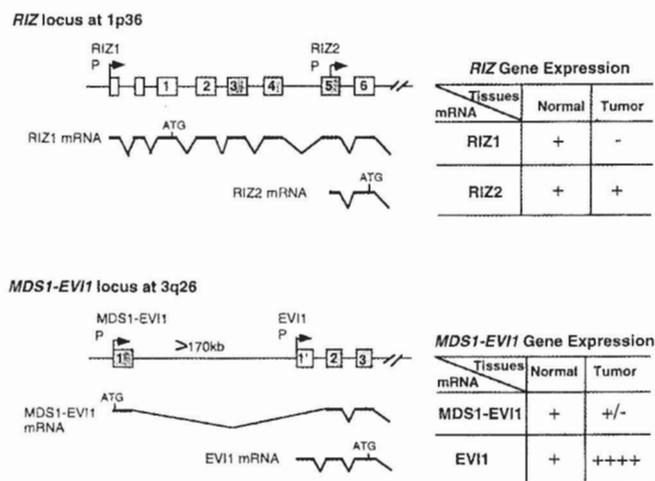


Fig. 5. yin-yang imbalance of *RIZ* and *MDS1-EVII* gene products in tumor cells. Partial genomic and mRNA structures (PR domain region only) of the 5'-end of *RIZ* and *MDS1-EVII* genes are shown schematically. P: promoter. Open boxes represent exons. The shaded boxes represent PR domain exons. Thin lines represent introns. Thick horizontal lines designate mRNA. The expression of *MDS1-EVII* in tumors is likely to be reduced in dosage (+/-) due to the allelic inactivation by translocation.

tumor prone (Steele-Perkins, G., Jiang, G-L., Yu, J.X., Yang, X-H., Liu, J-S., Bronson, R., and Huang, S., manuscript in preparation). Thus, for all practical purposes, RIZ1 represents a bona-fide tumor suppressor.

BLIMP1 in driving cell differentiation and repressing c-myc expression

BLIMP1 was isolated based on its high-level expression in differentiated cells versus proliferating nondifferentiated cells (Turner et al., 1994). Consistent with its induction in differentiated cells, *BLIMP1* acts like a master gene in controlling cell differentiation. Overexpression of *BLIMP1* can drive B-cell maturation into plasma cell. *BLIMP1* is a DNA-binding site-specific transcriptional repressor first described for the beta-interferon gene (Keller and Maniatis, 1991). The similarity of its DNA binding site to the repressor element found in the *c-myc* oncogene promoter led to the finding that *BLIMP1* is a repressor of *c-myc* transcription (Lin et al., 1997). Overexpression of *BLIMP1* can cause reduction of endogenous *c-myc* mRNA levels accompanied by terminal cell differentiation or apoptosis, depending on the cell type used.

The capacity of *BLIMP1* in driving cell differentiation, apoptosis, and repressing *c-myc* expression suggests a potential for *BLIMP1* in tumor suppression. Also consistent with this, *BLIMP1* maps to the YAC contig map of the human genome near the marker D6S447 on chromosome band 6q21-q22.1 (Mock et al., 1996). This region is commonly deleted in several types of human cancers including gastric carcinoma (Queimado et al., 1995), melanoma (Millikin et al., 1991; Thompson et al., 1995), and B-cell non-Hodgkin lymphomas (B-NHL) (Jonveaux and Berger, 1990; Levine et al., 1990; Schouten et al., 1990; Gaidano et al., 1992). It remains to be determined whether *BLIMP1* inactivation may occur in human cancers and correlate with *c-myc* overexpression.

Genetic alterations of ALL-1 in acute leukemia

Given that the PR domain is negatively involved in tumorigenesis, similar roles may be expected of the related SET domain. The best studied SET domain gene in the context of cancer is the *ALL-1* gene on human chromosome 11q23 that is involved in human acute leukemia through chromosome translocations or partial tandem duplications (Djabali et al., 1992; Gu et al., 1992; Tkachuk et al., 1992). *ALL-1* is an extremely large zinc finger protein of 3969 residues. The SET domain locates at the C-terminal end of the protein (residue 3840-3969). The chromosome translocations result in expression of chimeric proteins composed of the N-terminal ~1300 residues of *ALL-1* linked to a C-terminal polypeptide encoded by any (>25) of the partner genes. Because the partner genes fused to *ALL-1* are diverse, ranging from transcription factors to proteins

involved in signal transduction, the underlying mechanism for *ALL-1* in tumorigenesis is unknown. It seems implausible that each of the fusion genes in and of itself is oncogenic which would imply multiple distinct oncogenic mechanisms associated with *ALL-1* translocation.

It is reassuring to note that none of the overexpressed fusion proteins of *ALL-1* contains the SET domain. This observation suggests a possible negative selection for SET function in tumor cells, reminiscent of the PR domain. There are several lines of evidence that the SET-minus chimeric proteins exert a dominant negative effect on the normal *ALL-1* protein encoded by the intact allele present in the leukemic cells (Prasad et al., 1994; Schichman et al., 1994; Arakawa et al., 1998). Also, *ALL-1* inactivation may be involved in solid tumors (Baffa et al., 1995). The balance of the evidence favors *ALL-1* as a tumor suppressor.

Conclusions

A large body of evidence suggests the yin-yang hypothesis of PR genes in tumorigenesis. PR-plus product appears tumor suppressive whereas PR-minus product oncogenic. Proper balance of the two products may be key to maintaining normal cell homeostasis while an imbalance of the two may lead to cell transformation. The imbalance could be caused by inactivation of PR-plus product or activation of PR-minus product or both (Fig. 5). PR proteins may function in chromatin-mediated control of gene expression as inferred from the relationship with SET proteins. Chromatin regulation may represent a drastic functional difference between the PR-plus product and the PR-minus product of a PR gene, which may underlie their apparently opposite roles in tumorigenesis. PR family represents a new class of tumor suppressors, which rarely suffer intragenic mutations except in the PR domain. Loss of gene expression appears to be the common form of inactivation. There may be a good reason for this, i.e. the need for the presence of PR-minus products in tumor cells. All known PR genes map to end regions of chromosomes which are inherently unstable and commonly altered in cancer cells. Future studies on gene expression in relation to chromosome instability may shed light on the mechanisms of PR gene inactivation in cancer. Knock-out mice models with PR-plus but not PR-minus products ablated should provide a good model to prove that the commonly observed loss of PR-plus products in human cancers is causal to malignancy. Methods that can restore the normal yin-yang balance of PR gene products may be useful in treating human cancers.

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