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PRR5 encodes a conserved proline-rich protein predominant in kidney: analysis of genomic organization, expression, and mutation status in breast and colorectal carcinomas☆

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Abstract

Loss of heterozygosity on chromosome 22q13.31 is a frequent event during human breast and colorectal carcinogenesis. Herein we characterize a novel gene at chromosome 22q13.31 designated *PRR5*. Alternative promoter usage and splicing converge to generate five PRR5 transcript variants with maximum mRNA expression in kidney. In vitro transcription/translation demonstrated that the five variants generate three protein isoforms differing in their N-terminal length. Mutational analysis of *PRR5* in human breast and colorectal tumors did not reveal somatic mutations. However, mRNA expression analyses revealed *PRR5* overexpression in a majority of colorectal tumors but substantial downregulation of *PRR5* expression in a subset of breast tumors and reduced expression in two breast cancer cell lines. Treatment with trichostatin A increased PRR5 mRNA levels in BT549 and MDA-MB-231 cells, whereas 5′-aza-2′-deoxycytidine induced expression in MDA-MB-231 cells only. Thus, *PRR5* may represent a potential candidate tumor suppressor gene in breast cancer.
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Human carcinogenesis proceeds through the step-wise acquisition of genetic alterations that results in the production of neoplastic cells with increased malignant potential [1–5]. Chromosomal instability is one feature of carcinoma cells that is linked to properties of tumor cells such as enhanced proliferation and survival, increased rates of migration and invasion, and capacity to stimulate neoangiogenesis [1–5]. These cellular properties are often associated with the activation or overexpression of genes that reside within amplified regions of genomic DNA [6] and also with the inactivation of genes residing within regions of DNA copy loss (tumor suppressor genes) [2–5]. Tumor suppressor genes play critical roles in both the initiation and the progression of human carcinomas and are commonly inactivated by deletion [7,8], point mutation [9], or promoter hypermethylation with subsequent silencing of gene expression [10,11]. Any combination of the above three mechanisms may occur within

Abbreviations: 5azadC, 5′-aza-2′-deoxycytidine; CHTN, cooperative human tissue network; DME, Dulbecco's minimal essential; EST, expressed sequence tag; LOH, loss of heterozygosity; Q-PCR, quantitative real-time PCR; RACE-PCR, rapid amplification of cDNA ends-PCR; RT-PCR, reverse transcriptase-PCR; TSA, trichostatin A; UTR, untranslated region.

☆ Sequence data from this article have been deposited with the GenBank Data Library under Accession Nos. TPA: BK005634–BK005639.

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tumor cells to achieve biallelic tumor suppressor gene inactivation, which is transmissible to daughter progeny, in accordance with the Knudsen "two hit" hypothesis [12]. Given that inactivation of one allele of a tumor suppressor gene by point mutation is often accompanied by deletion of the second allele, studies of loss of heterozygosity in tumor cell genomes have been used to determine the location of potential tumor suppressor genes [13–15].

Loss of heterozygosity on the long arm of chromosome 22 has frequently been reported in many human cancer types, including adenocarcinoma (breast [16,17], colorectal [18,19], ovarian [20]), squamous cell carcinoma (head and neck [21,22], oral [23], esophageal [24]), thyroid cancer [25], astrocytoma [26], and Wilms tumor [27]. We have defined previously a minimal region of allelic loss of approximately 1 Mb (located between microsatellite markers *D22S1171* and *D22S928*) at chromosome band 22q13.31 in about 25% of sporadic primary colorectal tumors [18], which has been confirmed by another group [19]. The same minimal region of loss was subsequently delineated in primary breast tumors [16], in which 55% of cases examined harbored loss of heterozygosity at this locus. These data indicate that a tumor suppressor gene for both breast and colorectal cancer may reside within this interval.

Here we describe the identification and characterization of a novel gene (*PRR5*) positioned centromeric to *ARHGAP8* [28] at chromosomal band 22q13.31 that is predicted to encode a proline-rich protein of approximately 42 kDa. Our analyses demonstrate that use of alternative promoters and alternative splicing converge to generate five PRR5 mRNA transcript variants predicted to encode three protein isoforms. The potential involvement of PRR5 in breast and colorectal tumorigenesis was assessed through analysis of its mutation status and mRNA expression in primary breast and colorectal cancers.

Results

Organization of the PRR5 gene and protein

A candidate gene approach to the identification of the putative breast and colorectal cancer tumor suppressor gene located at chromosome 22q13.31 was undertaken. The *PARVB* and *PARVG* genes encode closely related actin-binding proteins and lie head to tail close to the centromeric boundary of the minimal region of deletion at 22q13.31 [29]. The *ARHGAP8* [28] (encoding a RHO GTPase activating protein) and *LOC112885* (predicted to encode a helicase) genes lie tail to tail close to the telomeric boundary. In between these two pairs of genes, four novel genes are predicted to reside. One of these genes, designated *PRR5* (approved Human Genome Nomenclature Committee symbol), was selected for characterization due to its location immediately centro-

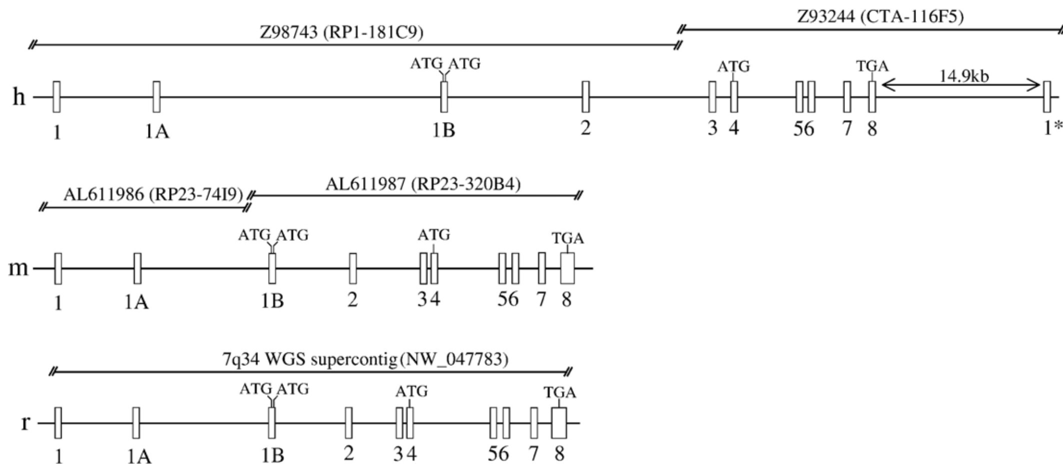

Fig. 1. Organization of the human (h), mouse (m), and rat (r) *PRR5* genes. Exons (not drawn to scale) are indicated by open vertical boxes and their positions were determined as described in Materials and methods. Introns are drawn to scale. The corresponding genomic clones (GenBank accession no. followed by clone I.D.) are shown above the genes. Human *PRR5* resides at chromosome 22q13.31, mouse *PRR5* on chromosome 15, region E2 and rat *PRR5* at 7q34. Rat PRR5 cDNAs containing exon 1 or exon 1A were not identified in the databases, and thus the sequences and locations of rat *PRR5* exon 1 and exon 1A were inferred by alignment of the rat chromosome 7q34 contig with mouse exon 1 and exon 1A sequences, respectively. In-frame putative initiation methionine residues (ATG) are shown in exon 1B and exon 4. The stop codon (TGA) lies in exon 8 of human and mouse and rat *PRR5*. Exon 1* corresponds to the *ARHGAP8* gene.

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