SHORT COMMUNICATION

Plasmacytoma-Associated Neuronal Glycoprotein, *Pang,* Maps to Mouse Chromosome 6 and Human Chromosome 3

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THIS PAPER IS DEDICATED TO THE MEMORY OF WES MCBRIDE, WHOSE UNTIMELY DEATH OCCURRED DURING THE COURSE OF THESE STUDIES

A new member of the immunoglobulin/fibronectin superfamily of adhesion molecules, *Pang* (plasmacytoma-associated neuronal glycoprotein), was recently isolated from a plasmacytoma. In previous studies, *Pang* was found to be normally expressed in the brain and ectopically activated by intracisternal A-type particle long terminal repeats in plasmacytomas. In this study, *Pang* was initially mapped to mouse Chr 6 by somatic cell hybrid analysis and further positioned on the chromosome between *Wnt7a* and *Pcp1*. Southern blot analysis of human–rodent somatic cell hybrids together with predictions from the mouse map location indicate that human PANG is located at 3p26.

Pang, a gene encoding a neuronal adhesion molecule, was isolated as a plasmacytoma-specific transcript utilizing an RT-PCR-based strategy in an attempt to isolate Myc-like genes (3). *Pang* is a member of the immunoglobulin/fibronectin superfamily of adhesion molecules; its closest relatives, TAG-1 and F11, promote axon growth and migration (2, 5). The normal site of *Pang* expression is the brain, where it was detected as 4.0- and 6.1-kb RNAs on Northern blots; *Pang* was not detected in any other normal tissues. Abnormally sized *Pang* transcripts were uniquely found in murine plasmacytomas (3). *Pang* is ectopically activated by intracisternal A-type particle (IAP) long terminal repeats (LTRs) in murine plasmacytomas; 30 of 50 plasma cell tumors expressed a 3.6-kb Pang transcript, and 80% of the plasmacytomas expressing IAP LTRs were *Pang* positive (3). In addition to the predominant 3.6-kb transcript, there were a number of smaller and larger transcripts that were tumor cell line specific (3). *Pang* is not likely to be involved in tumor establishment since its expression was absent in 40% of the plasmacytomas examined; however, this does not rule out the possibility that *Pang* could contribute to progressive, terminal phases of tumorigenesis. Genes on mouse Chrs 1 and 4 have been implicated in the genetic control of plasmacytoma susceptibility in BALB/c mice (13). To examine the relationship between *Pang* and genetic susceptibility to mouse plasmacytomagenesis, we determined the chromosomal locations of *Pang* in mouse and human.

Mouse *Pang* was initially mapped by Southern blot analysis (12) of a panel of *Hin*dIII-digested Chinese hamster × mouse somatic cell hybrid DNA in 17 somatic cell hybrids (described in Ref. 6) with a 1.5-kb mouse cDNA probe (3) specifying the complete *Pang* open reading frame. This mouse cDNA for *Pang* hybridizes to four *Hin*dIII fragments in BALB/c (9.7, 9.6, 7.0, and 6.4 kb) and hamster (18, 16, 5.8, and 3.8 kb). The BALB/c *Hin*dIII fragments cosegregated with mouse Chr 6 (0% discordancy) in the somatic cell hybrids (data not shown).

Four different patterns of hybridization to *Pang* were found with *Bam*HI-digested DNA from a variety of inbred strains of mice: (i) 28-, 18-, and 15-kb fragments were found in A/J, ABP/Le, AKR/A, BALB/cAnPt, BALB/cJ, C3H/HeN HSD, DBA/2J, DBA/2N, 020/A, and 129/J; (ii) 16- and 15-kb fragments were found in C57BL/6N and C57BL/10SnJ; (iii) 17-, 16-, and 15-kb fragments were found in NZB/BINJ, NZW, and SWR/J; and (iv) a 15.5-kb fragment was found in *Mus spretus.*

To determine the location of *Pang* on Chr 6, we performed Southern blots on genomic DNA (6) from two sets of backcross progeny: (i) (NFS/N or C58/J × *Mus musculus musculus*)F1 × *M. musculus musculus* and (ii) (NFS/N × *M. spretus*)F1 × *M. spretus* or C58/J (7). In the *musculus* cross, *Bgl*II fragments hybridizing to *Pang* were polymorphic between NFS/N or C58/J (9.0, 7.2, 6.3, 6.0, 4.8 kb), and *M. m. musculus* (9.7, 7.2, 6.3,

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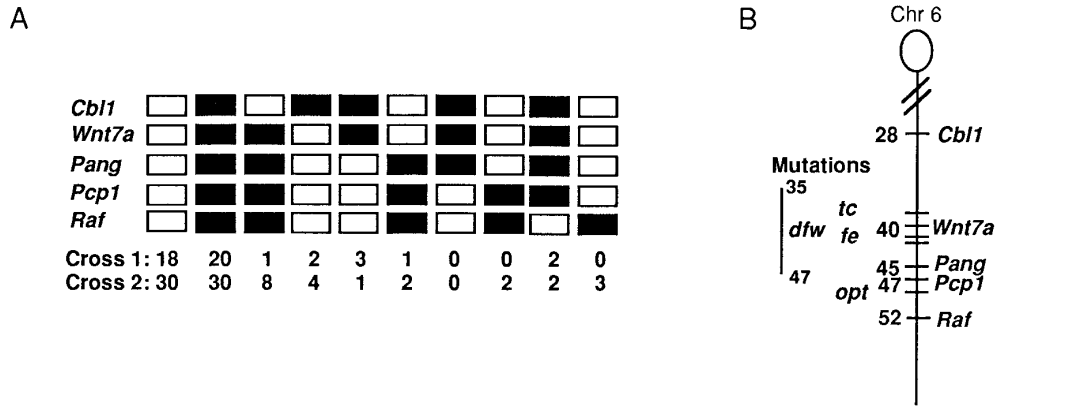

Cbl1 **-- 15/129, 11.6 + 3.6 --** ***Wnt7a*** **-- 7/129, 5.4 + 2.0 --** ***Pang*** **-- 2/129, 1.6 + 1.09 --** ***Pcp1*** **-- 7/129, 5.4 + 2 --** ***Raf1***

Wnt7a **-- 7/144, 4.9 + 1.8 --** ***Pang*** **-- 4/144, 2.8 + 1.4 --** ***Pcp1***

FIG. 1. Haplotype analysis of 129 backcross progeny from Cross 1 [(NFS/N or C58/J × *Mus musculus musculus*)F1 × *M. musculus musculus*] and Cross 2 [(NFS/N × *Mus spretus*)F1 × *M. spretus* or C58/J]. The loci followed in the crosses are indicated to the left. Positions of mutations were taken from the mouse Chr 6 report and are given to the left of the map. Each column represents the chromosome inherited in the backcross progeny; the number of progeny exhibiting each type of chromosome is listed at the bottom. The black squares represent either the NFS/N or the C58/J allele, and the open squares represent the *musculus* or *spretus* allele. Gene orders, recombination fractions, and distances (in centimorgans) between markers are indicated just below the figure. An additional 15 progeny were typed for alleles at *Wnt7a, Pang,* and *Pcp1;* distances between these markers based on the additional data are given at the bottom of the figure.

6.0, 4.8 kb); in the *spretus* cross, *Hin*dIII fragments were polymorphic between NFS/N or C58/J (14.5, 9.6, 7.0, 6.4 kb) and *M. spretus* (20.0, 14.0, 9.3 kb). These *Pang* fragments were mapped relative to the following molecular markers in both crosses: *Cbl1* (Casitas B lineage lymphoma), *Wnt7a* (wingless-related MMTV integration site 7A), *Pcp1* (purkinje cell protein 1), and *Raf1* (ras-related fibrosarcoma oncogene). Restriction fragment variants for these markers have been previously described (1).

Haplotype analysis of 129 progeny from the two crosses (Fig. 1) allowed the positioning of *Pang* with respect to the other four Chr 6 markers. Additional 2 × 2 data available for *Wnt7a, Pang,* and *Pcp1* yielded the following recombination fractions and distances in centimorgans: centromere–*Wnt7a*–7/144, 4.9 ± 1.8–*Pang*–4/144, 2.8 ± 1.4–*Pcp1*. A composite map of Chr 6, along with positions of morphological mutations mapping in the interval between *Wnt7a* and *Pcp1,* is shown in Fig. 1. Positions of morphological markers are taken from the current Chr 6 report (4).

Human PANG was localized by Southern blot analysis (10) of a panel of *Eco*RI-digested human–rodent somatic cell hybrid DNA (described in (8, 9) with the same 1.5-kb mouse PANG cDNA probe used above). The membranes were hybridized (42°C in 30% formamide) and washed (0.1× SSC at 42°C) at moderate stringency allowing about 20–25% sequence divergence. The largest (8.2 kb) hybridizing band in human DNA was readily distinguished from hybridizing bands in rodent DNA, but the two smaller human bands could not be resolved and mapped in the hybrids. The gene could be unambiguously assigned to human chromosome 3 (Table 1), and it segregated discordantly (>18%) with all other human chromosomes. The gene was further localized on the short arm of Chr 3 by examination of two hybrids containing spontaneous breaks or deletions involving this chromosome. One hybrid contained a large deletion in the short arm with loss of distal 3p markers RAF1 (3p25), THRB (3p24.1–p22), and ACY1 (3p21), but retention of DNF15S2 (3p21.2–p21.3), CCHL1A2 (3p21), and two other more centromeric 3p markers and all loci on 3q; PANG was retained in this hybrid. The other hybrid contained a break in proximal 3q with retention of long arm markers and loss of all short arm markers, and the human PANG gene was absent from this hybrid. These results indicate that the gene must be located in the region 3p21–cen or at 3p26.

No restriction fragment length polymorphisms were detected with the PANG probe on examination of DNA from peripheral leukocytes of 10 unrelated individuals separately digested with 12 different restriction enzymes (*Eco*RI, *Bam*HI, *Hin*dIII, *Xba*I, *Sac*I, *Taq*I, *Pvu*II, *Pst*I, *Bgl*II, *Msp*I, *Eco*RV, and *Kpn*I).

The chromosomal location of *Pang* on Chr 6 in the mouse precludes it as a candidate gene for the plasmacytoma susceptibility genes identified on Chrs 1 and 4 (13). *Pang* was not polymorphic between the BALB/c and DBA/2 mice used in the tumor susceptibility linkage studies; it will be of interest to reexamine the region of Chr 6 where *Pang* is located among the backcross progeny to look for associations of minor susceptibility/resistance genes with this region of the genome. In addition, tumors induced in F1 hybrid mice will be examined by SSLP (simple sequence length polymorphism) analysis for loss of heterozygosity in this region of Chr 6.

The location of *Pang* approximately 45 cM from the centromere, as determined by comparison with the consensus map of Chr 6 (4), places this gene within 5–8

TABLE 1

Segregation of the PANG Gene with Human Chromosome 3

Human chromosome	Gene/chromosome +/+	+/−	−/+	−/−	Percentage discordancy
1	22	12	9	49	23
2	18	16	9	49	27
3	34	0	0	58	0
4	32	2	25	33	29
5	21	13	4	54	18
6	25	9	24	34	36
7	14	20	27	31	51
8	23	11	14	44	27
9	5	29	8	50	40
10	16	18	4	54	24
11	21	13	12	46	27
12	22	12	17	41	32
13	15	19	20	38	42
14	15	19	29	29	52
15	17	17	30	28	51
16	11	23	16	42	42
17	22	12	35	23	51
18	24	10	24	34	37
19	21	13	8	50	23
20	24	10	16	42	28
21	30	4	29	29	36
22	17	17	11	47	30
X	24	10	22	36	24

Note. The PANG gene was detected as 4.3-, 5.2-, and 8.2-kb bands in *Eco*RI digests of human DNA and human–rodent somatic hybrid cell DNA after Southern hybridization with a 1.5-kb mouse cDNA insert probe. The 8.2-kb cross-hybridizing human band was well resolved from hybridizing 1.9-, 2.2-, 3.8-, 4.2-, 5.0-, 6.4-, and 18-kb or 1.0-, 1.7-, 2.2-, 2.3-, 2.7-, 3.1-, and 4.9-kb bands in *Eco*RI-digested Chinese hamster and mouse DNA, respectively. Detection of the human gene is correlated with the presence or absence of each human chromosome in the group of somatic cell hybrids. Discordancy represents the presence of the gene in the absence of the chromosome (+/−) or the absence of the gene despite the presence of the chromosome (−/+), and the sum of these numbers divided by the total number of hybrids examined (×100) represents the percentage of discordancy. The human–hamster hybrids consisted of 29 primary hybrids and 11 subclones (14 positive of 40 total), and human–mouse hybrids contained 20 primary clones and 32 subclones (20 positive of 52 total).

cM of the morphological mutations *tc* (truncated), *fe* (faded), *opt* (opisthotonous), and *dfw* (deaf waddler). Given its predominant expression in the brain and putative role as a neuronal adhesion molecule, it becomes an interesting candidate to examine in the neurological mutants *opt* and *dfw*. This region of mouse Chr 6 shares linkage homology with human 3p25–p26; this suggests that the likely location of human PANG on 3p is at 3p26. Human diseases linked to the 3p26 region include renal cell carcinoma and xeroderma pigmentosum, complementation group C (11), while no known neurological syndromes have been linked to this region of human 3p.

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REFERENCES

1. Adamson, M. C., Dennis, C., Delaney, S., Christiansen, J., Monkley, S., Kozak, C. A., and Wainwright, B. (1994). Isolation and genetic mapping of two novel members of the murine Wnt gene family, Wnt11 and Wnt12, and the mapping of Wnt5A and Wnt7A. *Genomics* 24: 9–13.
2. Brummendorf, T., Wolff, J. M., Frank, R., and Rathjen, F. G. (1989). Neural cell recognition molecule F11: Homology with fibronectin type III and immunoglobulin type C domains. *Neuron* 2: 1351–1361.
3. Connelly, M. A., Grady, R. C., Mushinski, J. F., and Marcu, K. B. (1994). PANG, a gene encoding a neuronal glycoprotein, is ectopically activated by intracisternal A-type particle long terminal repeats in murine plasmacytomas. *Proc. Natl. Acad. Sci. USA* 91: 1337–1341.
4. Elliott, R. W., and Moore, K. J. (1994). Mouse Chromosome 6. *Mamm. Genome* 5: S79–S103.
5. Furley, A. J., Morton, S. B., Manalo, D., Karagogeos, D., Dodd, J., and Jessell, T. M. (1990). The axonal glycoprotein TAG-1 is an immunoglobulin super family member with neurite outgrowth-promoting activity. *Cell* 61: 157–170.
6. Hoggan, M. D., Halden, N. R., Buckler, C. E., and Kozak, C. A. (1988). Genetic mapping of the mouse c-fms protooncogene to chromosome 18. *J. Virol.* 62: 1055–1056.
7. Kozak, C. A., Peyser, M., Krall, M., Mariano, T. M., Kumar, C. S., Pestka, S., and Mock, B. A. (1990). Molecular genetic markers spanning mouse chromosome 10. *Genomics* 8: 519–524.
8. McBride, O. W., Battle, J., Hollis, G. F., Swan, D. C., Siebenlist, U., and Leder, P. (1982). Localization of human variable and constant region immunoglobulin heavy chain genes on subtelomeric band q32 of chromosome 14. *Nucleic Acids Res.* 10: 8155–8170.
9. McBride, O. W., Hieter, P. A., Hollis, G. F., Swan, D. C., Otley, M. C., and Leder, P. (1982). Chromosome localization of human kappa and lambda immunoglobulin light chain constant region genes. *J. Exp. Med.* 155: 1480–1490.
10. McBride, O. W., Pirtle, I. L., and Pirtle, R. M. (1989). Localization of three DNA segments encompassing tRNA genes to human chromosomes 1, 5, and 16: Proposed mechanism and significance of tRNA gene dispersion. *Genomics* 5: 561–573.
11. McKusick, V. A. (1994). "Mendelian Inheritance in Man," Johns Hopkins Univ. Press, Baltimore.
12. Mock, B. A., D'Hoostelaere, L. A., Matthal, R., and Huppi, K. (1987). A mouse homeo box gene, *Hox-1.5*, and the morphological locus, *Hd*, map to within 1 cM on chromosome 6. *Genetics* 116: 607–612.
13. Mock, B. A., Krall, M. A., and Dosik, J. K. (1993). Genetic mapping of tumor susceptibility genes involved in mouse plasmacytomagenesis. *Proc. Natl. Acad. Sci. USA* 90: 9499–9503.