

LACK OF PHOSPHOLIPASE A₂ MUTATIONS IN NEUROBLASTOMA, MELANOMA AND COLON-CANCER CELL LINES

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A candidate murine tumor-suppressor gene, *Mom1*, has been identified as the secretory phospholipase A₂ (GDB nomenclature: *PLA2G2A*) gene. Evidence suggests that *PLA2G2A* functions as a tumor-suppressor because mice lacking *PLA2G2A* expression demonstrate increased colonic polyposis. The human homologue of *PLA2G2A* has been mapped to chromosome 1p36, a region frequently implicated in the pathogenesis of neuroblastoma, colon cancer and melanoma. We identified 2 alterations in the *PLA2G2A* gene in a single neuroblastoma cell line out of 20 examined; however, we found no mutations in 24 melanoma cell lines, 12 lymphoblastoid cell lines from patients having chromosome 1-linked familial melanoma and 10 colon cancer cell lines. Secretory phospholipase A₂ is unlikely to play a significant role in the pathogenesis of these tumors. *Int. J. Cancer* 72:337–339, 1997.

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The short arm of chromosome 1 includes a region lost in a substantial portion of a variety of neoplasms. The best characterized alterations have been described in neuroblastoma, in which the most frequent cytogenetic abnormality described is loss of chromosome 1p (Brodeur *et al.*, 1981). A constitutional deletion of chromosome 1p36 has been characterized in a child with neuroblastoma, suggesting the presence of a tumor-suppressor locus at this site (Biegel *et al.*, 1993). Extensive analysis of 1p using polymorphic microsatellite markers also has suggested that this is the most consistently involved region and has narrowed the interval to 1p36.2–1p36.3 (White *et al.*, 1995). The gene or genes involved in these chromosomal alterations remain to be identified.

Chromosome 1p36 is also altered in other neural crest malignancies. In melanoma, the cytogenetically most frequently abnormal chromosome region is 1p (Fountain *et al.*, 1990). Loss of heterozygosity (LOH) in this region also has been examined, and, as for neuroblastoma, the most commonly involved region includes 1p36 (Dracopoli *et al.*, 1989). Moreover, linkage has been established between polymorphic markers on chromosome 1p and inherited susceptibility to melanoma in a subset of melanoma kindreds, further supporting the localization of a melanoma-associated tumor-suppressor there (Goldstein *et al.*, 1993). This finding has been a subject of controversy though. Five additional studies examining the transmission of hereditary melanoma have been unable to substantiate the 1p36 linkage (reviewed in Haluska and Housman, 1995). The 9p21-linked melanoma susceptibility gene was identified with the isolation of the *p16/CDKN2* gene (Nobori *et al.*, 1994; Kamb *et al.*, 1994) and the elucidation of mutations in this gene in 9p21-linked families (Hussussian *et al.*, 1994). It is noteworthy, however, that families that had been previously analyzed and shown to exhibit linkage to chromosome 1p do not carry *p16/CDKN2* mutations, though in some of them, a mutation in cyclin-dependent kinase 4 (CDK4) has been identified (Hussussian *et al.*, 1994). This strongly suggests that a chromosome 1 melanoma tumor-suppressor may yet be identified.

The third cancer that demonstrates LOH on chromosome 1p is colon cancer. Cytogenetic and molecular studies of human colon cancers have revealed alterations of chromosome 1p, with LOH for 1p markers in over 40% of tumors tested (Leister *et al.*, 1990). In

addition, studies of the murine model for familial adenomatous polyposis coli (APC) have identified a potential tumor-suppressor locus that maps to 1p36. The *Min* mouse carries a germ-line *Apc* mutation; heterozygous animals develop multiple polyposis and colorectal cancer. A locus that modifies this phenotype, *Mom1*, was identified (Dietrich *et al.*, 1993) and the responsible gene isolated (MacPhee *et al.*, 1995). The gene is secretory phospholipase A₂ (*PLA2G2A*). Mice null for this gene demonstrate an augmented polyposis phenotype; the gene thus functions as a tumor-suppressor. The murine *PLA2G2A* maps to a region of mouse chromosome 4 syntenic with human 1p36, and the human homologue has been mapped to 1p35–36.1 (Praml *et al.*, 1995). Thus, this gene is a candidate for one of the tumor-suppressor loci on chromosome 1p36.

We thus analyzed the human *PLA2G2A* in human cell lines from neuroblastomas, melanomas, 1p36-linked melanoma families and colon cancers. We identified 2 neuroblastoma cell lines, derived from a single neuroblastoma, that carry 2 alterations in the *PLA2G2A* gene.

MATERIAL AND METHODS

Cell lines

Melanoma tumor cell lines have been described previously (Fountain *et al.*, 1992). Lymphoblastoid cell lines established from families segregating familial melanoma and demonstrating chromosome 1p linkage also have been described previously (Bale *et al.*, 1989). The colon-cancer cell lines tested included LS174T, SW403, SW48, SW480, SW948, COLO320 DM, WiDr, SW620, HT29 and SK-CO-1 and were obtained from the ATCC (Rockville, MD). The SK-N-SH neuroblastoma cell line also was obtained from the ATCC; other lines were described previously (Thiele *et al.*, 1987).

PCR analyses

High-m.w. DNA was extracted from tissue culture samples using standard procedures. DNA was subjected to single-strand conformation polymorphism analysis (SSCP) for the detection of sequence alterations. Primers were constructed from previously published (Kramer *et al.*, 1990) sequences of the genomic structure of the human *PLA2G2A* gene as follows: 2418F, AGTCCATCTGCATTTGTCACCC; 2573R, ATGGGAGAGAAAACCTAAGGGAC; 266F, CCCATCACCAGACAACCTCCC; 2913R, GTCCTTTCAGCTC1CCAGCCC; 3065F, AGCTGTGGGACAAGAGCCCC; 3249R, CAAATGAGGGCCACTCGATGG; 5329F, GCCACTGAATATTAATAAACTCCC; 5566R, AAATTCAGCACTGGGTGGAAGG. PCR reactions were performed using end-labeled primers. Primer (40 pmol) was labeled using T4 polynucleotide kinase (New England Biolabs, Beverly, MA), and under standard conditions

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reactions were performed in an MJ thermocycler: 35 cycles of 95°C for 30 sec, 62°C for 30 sec, and 72°C for 1 min. Upon completion of the reactions, 25 µl of stop solution (95% formamide, 10 mM EDTA) were added. The reaction was heated to 95°C for 5 min, cooled immediately on ice for 5 min and then allowed to come to room temperature. For analysis, 2 µl of each sample were loaded on an MDE (FMC Bioproducts, Rockland, ME)/5% glycerol gel and run at 6.0 W at room temperature for approx. 12 hr. Gels were then dried and exposed to autoradiography film at room temperature for 2–12 hr.

DNA sequencing

Samples demonstrating an aberrant band by SSCP were selected for sequence analysis. PCR products were separated on a 1.5% low-melting-point agarose gel, excised and purified using b-agarase (New England Biolabs) according to the manufacturer's specifications. Purified PCR product was subject to cycle sequencing (fmol Sequencing Kit, Promega, Madison, WI) using end-labeled primer and analyzed on an 8% acrylamide gel. Finished gels were dried and autoradiographed at room temperature for 2–24 hr.

RESULTS AND DISCUSSION

The human secretory *PLA2G2A* gene consists of 5 exons; the first of these is not translated (Kramer *et al.*, 1990). We thus designed oligonucleotide primers to allow us to assess coding exons 2–5 by SSCP analysis. We examined neuroblastoma cell lines, melanoma cell lines, lymphoblastoid cell lines from unaffected individuals, lymphoblastoid cell lines from patients whose families carry malignant melanoma linked to chromosome 1 and colon cancer cell lines.

Under the conditions tested, no polymorphisms were detected in any of the samples examined in exon 4.

One neuroblastoma cell line, SK-N-SH, demonstrated SSCP shifts in 2 exons, exon 2 and exon 5 (Fig. 1). None of the other 102 samples examined exhibited shifts in either of these exons. We sequenced the purified PCR products to identify the basis of the SSCP shifts. The shift in the migration of the exon 2 product is due to a C-to-T alteration at nucleotide position 2500. This nucleotide lies 8 bp into the second intron of the *PLA2G2A* gene. The mutation is illustrated in Figure 2a. The exon 5 shift is due to a G-to-A alteration at nucleotide 5518, resulting in an arginine-to-histidine substitution of codon 143. This is illustrated in the sequence of the anti-sense strand as a C-to-T mutation in Figure 2b. Like the exon 2 shift, this mutation was not identified in any of the 102 additional samples tested. However, it was identified previously in a single colon cancer (Riggins *et al.*, 1995) and in that case determined to represent a germ-line, not a somatic, mutation.

The neuroblastoma SK-N-SH is heterozygous for both of these alterations. From the data so far obtained, it is not clear whether these mutations are present on the same chromosome or not; if they

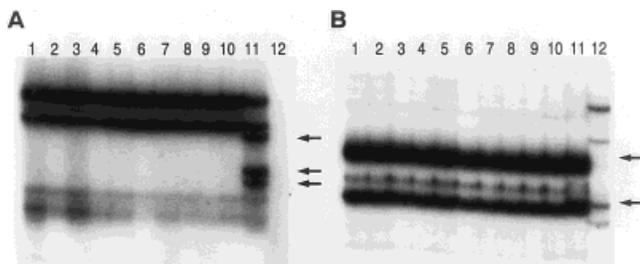


FIGURE 1 – SSCP demonstrating shifts in the neuroblastoma cell line SK-N-SH (lane 11) in exons 2 (a) and 5 (b). Lanes 1–10: colon cancer cell lines. Lane 12: negative (no DNA) control.

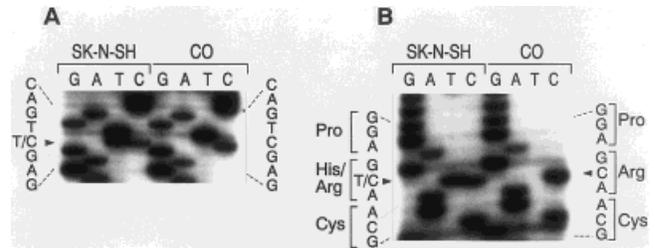


FIGURE 2 – Sequence analysis of SK-N-SH exons 2 (a) and 5 (b). The exon 2 C-to-T substitution occurs at position 2500, 8 bp into the second intron. The exon 5 alteration at nucleotide 5518 results in Arg143His. CO is a colon cancer cell line lacking SSCP abnormalities.

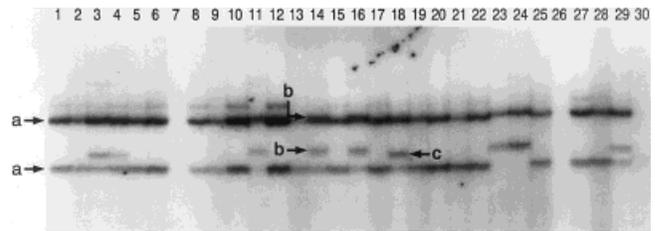


FIGURE 3 – SSCP analysis of 24 melanoma cell lines and 5 lymphoblastoid cell lines (lanes 1–29) demonstrating polymorphisms of the *PLA2G2A* exon 3. Allele a designates the pattern corresponding to the published (Riggins *et al.*, 1995) sequence. Alleles b and c are polymorphisms described in the text. Lane 30: negative (no DNA) control. PCR failed in lanes 7 and 26.

are, both copies of the *PLA2G2A* gene would be abnormal. Moreover, the functional consequences of these changes are unknown. The intronic mutation lies outside of the conserved splice donor recognition motif, and it may thus be an infrequent, silent polymorphism. The functional consequence of the Arg143His mutation is also unknown. It is noteworthy that this mutation has been detected only in malignancies. However, the lack of additional alterations in the panel of neuroblastoma cell lines examined suggests that these mutations are not a common feature of this tumor type and that *PLA2G2A* is not the target of chromosome 1 alterations in neuroblastoma.

Exon 3 demonstrated three alleles in the various samples examined (Fig. 3) by SSCP. PCR products from samples demonstrating each of the alleles, designated a, b and c in Figure 3, were purified and sequenced (data not shown). Allele a corresponds to the published *PLA2G2A* sequence. Allele b represents a C-to-T substitution at nucleotide 2793, a silent alteration in codon 44. This allele was present in 17 of 103 samples examined; its information content could not be estimated as many of the samples are aneuploid tumor cell lines. Allele c represents a G-to-C substitution at nucleotide position 2757; this is a silent alteration in codon 32. It was present on 21 of 103 samples. These alleles are thus frequent polymorphisms. Several studies have reported similar results (Riggins *et al.*, 1995; Spirio *et al.*, 1996).

The data presented here suggest that the *PLA2G2A* gene, despite its candidacy as the murine *Mom1* tumor-suppressor and its chromosome 1p36 localization, is not one of the potential tumor-suppressor loci implicated in neuroblastoma, melanoma, or colon cancer. The importance of the alterations of the gene described here in a single neuroblastoma cell line remains to be elucidated.

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