

Protein Tyrosine Phosphatase Genes Downregulated in Melanoma

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Phospho-tyrosine levels are increased in melanoma, apparently consistent with reports of elevated protein tyrosine kinase activity. Some protein tyrosine kinases are encoded by oncogenes and have been implicated in melanoma genesis. Decreased protein tyrosine phosphatase activity may also increase phospho-tyrosine. Protein tyrosine phosphatase genes are candidate tumor suppressors and loss of expression may contribute to melanoma genesis. Here we survey protein tyrosine phosphatase expression in pigment cells. Protein tyrosine phosphatase genes were cloned by reverse transcriptase polymerase chain reaction using degenerate primers based upon conserved sequences within the phosphatase catalytic domain. Reaction products were cloned and sequenced: 118 and 113 partial protein tyrosine

phosphatase products were isolated from normal melanocytes and melanoma cells, respectively. Northern blotting analysis was used to study expression of 15 protein tyrosine phosphatase genes. Expression of PTP- κ and PTP- π was absent or downregulated in more than 20% of melanoma cell lines and in some unmanipulated melanoma biopsies. These closely related enzymes are members of the 2B receptor protein tyrosine phosphatase family previously implicated in contact inhibition. Loss of protein tyrosine phosphatase expression may contribute to the abnormal tyrosine phosphorylation seen in melanoma; these genes are candidate tumor suppressors. Key words: melanocyte/metastasis/PTP- κ /PTP- π . *J Invest Dermatol* 117:1255–1260, 2001

Currently, there is no effective therapy for advanced melanoma (McMasters *et al*, 1999). Many studies have searched for qualitative differences in signal transduction pathways between normal and malignant cells in the hope that these might be exploited for the development of novel strategies for treatment. Recently, attention has focused on protein-tyrosyl phosphorylation, a key component of mammalian cellular signaling pathways (Hunter, 1998). Protein tyrosine phosphatases and kinases (PTP and PTK) mediate signaling for multiple processes, including growth, survival, differentiation, migration, and adhesion (Van der Geer *et al*, 1994; Byon *et al*, 1997). These enzymes catalyze the addition (kinases) or removal (phosphatases) of phosphate on tyrosyl residues in substrate proteins. Certain PTK family members are oncoproteins and mediate tumorigenesis in common solid cancers (Hunter, 1998). Melanoma cells contain increased phospho-tyrosine residues compared to their normal cellular counterparts (Halaban *et al*, 1992), apparently reflecting overexpression of PTK (Halaban *et al*, 1992; de Wit *et al*, 1992). Although PTK have been extensively studied in melanoma, PTP have received little attention. Long suspected to include the products of tumor

suppressor genes (Sun and Tonks, 1994; Hunter, 1998), PTP may play a role in melanoma genesis; increased phospho-tyrosine levels in melanoma may result from a loss of PTP expression.

Increased PTK activity is believed critical for melanoma initiation and progression (Albino and Fountain, 1993; Chin *et al*, 1998). This assumption is based upon two types of data. First, overexpression of some PTK has resulted in melanomas in experimental animals; this includes X-Mrk in *Xiphophorus* (Wellbrock *et al*, 1998), and Ret (Iwamoto *et al*, 1991) and HGF (Otsuka *et al*, 1998), a ligand for Met, in transgenic mice. Human melanocytic cells do not express RET, however (Easty *et al*, 1995b), and the relationship of such models to human melanoma remains uncertain. In addition, transgenic animal cells express very high levels of PTK, which are not reported for any PTK in human melanoma cells. Second, increased expression of multiple PTK genes occurs in melanoma (Easty and Bennett, 2000). Increases are reported to occur, however, for multiple PTK genes, each in a subset of melanomas (Gutman *et al*, 1994; Quong *et al*, 1994). Moreover, immunohistochemical studies of specific PTK in melanoma biopsies have been disappointing; no single, common change (equivalent to, say, ERB-B2 in breast cancer) has been identified (Easty and Bennett, 2000). Nonetheless, we find particular PTK are likely to contribute to melanoma progression: EPH-A2 in growth (Easty *et al*, 1995a), focal adhesion kinase in adhesion (Maung *et al*, 1999), and KDR in metastasis¹ (reviewed by Easty and Bennett, 2000). Taken together, these data imply a role

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Abbreviations: PTK, protein tyrosine kinase; PTP, protein tyrosine phosphatase.

¹Nesbit *et al* (submitted for publication).

for tyrosine phosphorylation in melanoma genesis, although the mechanism remains uncertain.

Tyrosine phosphatases comprise cytoplasmic and receptor-like families; full sequences are available for more than 75 PTP, and the receptors have been divided into six main families (Neel and Tonks, 1997). Little is known concerning their expression or function in melanocytes. Tyrosine phosphatases are characterized by a conserved catalytic domain of about 240 amino acids containing a unique signature motif: (I/V)HCXAGXXR(S/T)G (where X indicates any amino acid); the cysteinyl residue represents the position for formation of a thio intermediate as part of the reaction mechanism (Stone and Dixon, 1994). In general PTP attenuate signals generated by PTK; they inhibit cell growth and transformation by PTK (Brown-Shimer *et al*, 1992; Zander *et al*, 1993). Importantly, total cellular PTP activity is 100–1000 times that of PTK (Sun and Tonks, 1994). This figure is an overview; it does not consider the activity or compartmentalization of individual PTP. In this regard, structural motifs within the intracellular PTP localize these gene products to specific cellular compartments and this is likely to determine substrate range (Neel and Tonks, 1997). Small alterations in PTK expression may not be functionally significant in the presence of normal levels of PTP activity. Furthermore, most studies of PTK expression in melanoma have found only small (less than 6-fold) increases in expression, and these often occur in advanced melanoma (Easty and Bennett, 2000). In the absence of PTK mutations (which have never been reported in human melanoma) a marked overexpression, perhaps of the order seen in animal models, might be required for transformation.

In order to learn more about PTP expression, we used reverse transcriptase polymerase chain reaction (RT-PCR) to isolate partial cDNAs from normal and malignant melanocytes. The highly degenerate oligonucleotide primers used were based upon conserved sequences from within the consensus catalytic domain of PTP. We find that melanocytic cells express a complex mixture of PTP genes. Northern blotting analysis was used to test 15 PTP genes in normal melanocytes and cell lines established from defined stages of melanoma progression. Two closely related genes, PTP- π and PTP- κ , members of one receptor PTP subfamily, were commonly downregulated in some melanoma cell lines and unmanipulated biopsies. Loss of PTP genes may provide an explanation for the increased amount of phospho-tyrosine reported in melanoma; such PTP genes are candidate melanoma suppressors.

MATERIALS AND METHODS

Melanoma specimens Tissue from malignant melanoma specimens was obtained as previously described (Florenes *et al*, 1992); biopsies were snap frozen in liquid nitrogen immediately following surgery.

Culture of melanocytes and melanoma cell lines Normal human melanocytes (FM1000, FM2056, FM2067) were isolated from neonatal foreskins as described previously (Hsu and Herlyn, 1998); one additional sample of melanocytes was from Clonetics (BioWhittaker, San Diego, CA). Melanocytes were maintained in melanocyte growth medium (MGM-3) containing insulin, hydrocortisone, basic fibroblast growth factor, and phorbol myristate acetate (Clonetics). The melanoma cell lines used here and their original sources have been described previously (Easty *et al*, 1993, 1995a, 1995b; Hsu and Herlyn, 1998). Cell lines WM9, WM239A, WM164, WM852, WM983A, WM1158, 451LU, DX3, A375M, and COLO832 were derived from metastases; WM35, WM793, WM1341B, WM1650, and SBcl₂ were from primary melanomas. Lines P1N1 and P2N1 were derived from WM35, WM793, and WM1431B. The three parental lines failed to form tumors, or were poorly tumorigenic (WM793) in animals. When inoculated in the presence of Matrigel, derivative cell lines were isolated after one (P1N1) or two (P2N1) passages in mice. These derivatives appeared more tumorigenic in animals than the parental lines and resembled cells derived from more aggressive vertical growth phase melanomas (Kobayashi *et al*, 1994). These cell lines were a gift from Prof. Robert Kerbel (Toronto). One early melanoma cell line (SBcl₂) was cultured in MCDB 153 with 20% L-15 medium and supplemented with 2 Mm Ca, 2% fetal bovine serum (FBS), and 5 μ g per ml insulin (Hsu and Herlyn, 1998). All melanoma cell lines were maintained in Dulbecco's modified

Eagle's medium supplemented with 5% FBS and glutamine and incubated with 10% CO₂, except where stated otherwise.

CDNA synthesis Poly(A⁺)-enriched RNA was isolated from normal melanocytes and a metastatic melanoma cell line (451LU) using a Fastrack 2.0 kit from Invitrogen. We prepared first strand cDNA using 5 μ g of poly(A⁺)-enriched RNA and a Superscript II kit from Gibco BRL as per the manufacturer's instructions.

Degenerate PCR Degenerate primers derived from sequences within the highly conserved region of the catalytic domain were used for PCR to amplify multiple members of the PTP gene family from normal melanocytes and 451LU melanoma cells. For PCR, degenerate sense [F1 and F2; encoding amino acids FWXMXW (single letter amino acid code; X, any amino acid)] and antisense [B1, B2, and B3; encoding HCSAG(I/V)] primers were used (Norris *et al*, 1997). Primer combinations F1 + B1, F2 + B2, and F1 + B3 were contained in a 50 μ l reaction with 1 μ l of the 451LU or melanocyte cDNA. The reaction mixture was subjected to the conditions previously described: 95°C for 4 min; then 95°C for 1 min, 37°C for 2 min, and 72°C for 3 min for three cycles; next 95°C for 1 min, 42°C for 2 min, and 72°C for 3 min for five cycles; next 95°C for 1 min, 47°C for 2 min, and 72°C for 3 min for five cycles; and finally 95°C for 1 min, 53°C for 2 min, and 72°C for 3 min for 27 cycles. The PCR yielded products of about 400 bp; these were subcloned into pGEM-T (Promega). In addition, specific primers were designed for PTP-1B, PTP-SIGMA, PTP-DELTA, PTP-ZETA, PTP-EPSILON, and PTP- β using a MacVector version 4.5 program. Purified PCR products were used for northern and southern blotting analysis (Easty *et al*, 1995a).

Subcloning of degenerate PCR products Approximately 25 ng of the PCR product was ligated to 50 ng of pGEM-T vector (Promega) using T4 DNA ligase; products were transformed into JM109 competent cells, and colonies were selected on LB-ampicillin plates.

DNA Sequencing For each clone, miniprep DNA was prepared as previously described (Easty *et al*, 1993), and PTP gene fragments in pGEM-T were amplified using M13For and M13Rev primers. Clones of the expected size were sequenced using an ABI PRISM Dye Terminator Cycle Sequencing Ready Reaction Kit and ABI PRISM 310 Genetic Analyzer. The resulting sequences were compared to the database using the NCBI Blast program.

Northern blotting analysis Poly(A⁺)-enriched RNA was prepared from cell lines as previously described (Easty *et al*, 1993); 2.5 μ g or 5 μ g was electrophoresed through a 1% agarose-formaldehyde gel and transferred to nitrocellulose membranes. Total RNA was isolated from melanoma biopsies as previously described (Florenes *et al*, 1992). Probes were labeled by random hexanucleotide priming; prehybridization and hybridization were all as before (Easty *et al*, 1995a). Products cloned from RT-PCR were used as probes for northern blotting (**Table I**); additional PCR products used as probes (PTP-BAS, PTP-LAR, PTP-H1, PTP-MEG1, TCELL-PTP, and PTP-SIGMA) were from normal human mammary epithelial cells.² The PCR products used as probes detected transcripts of the expected size(s); in addition, southern blotting analysis found single bands consistent with specific hybridization by probes. For some PTP probes multiple transcripts were detected. These blots were repeated with specific PCR products corresponding to regions that excluded the conserved catalytic region and the same transcript sizes were found, consistent with gene-specific hybridization. The integrity and loading of RNA from cell lines was determined by probing with glyceraldehyde phosphate dehydrogenase (GAPDH) and/or actin; for the unmanipulated melanoma biopsies, a specific 18S rRNA oligonucleotide was used for quantitation as previously described (Florenes *et al*, 1992). Laser densitometry was used to scan autoradiographs, and the mRNA index was calculated as previously described (Easty *et al*, 1995b). We regarded a difference in expression as significant whenever there was a 3-fold or greater difference compared to normal melanocytes for two or more of the cell lines tested (Easty *et al*, 1993).

Southern blotting analysis DNA was extracted from melanoma cell lines and control DNA from lymphoblastoid cell lines. Blotting was as previously described (Easty *et al*, 1995a). DNA (10 μ g) was digested with restriction enzymes, fractionated by agarose gel electrophoresis, and transferred to nylon membranes. Probes for PTP-LAR, PTP- κ , and PTP- π were isolated by PCR and used for southern blotting analysis.

²Rafferty *et al*: *Eur J Cancer* (submitted for publication).

Table I. Incidence of different PTP among sequenced PCR clones

Family	Phosphatase	Incidence ^a			
		Melanocytes		Melanoma	
		n	%	n	%
Receptors					
μ	κ	4	3.4	5	5.5
	π	2	1.7	0	0
	α	5	4.2	2	1.8
	β	4	3.4	3	2.7
	γ	0	0	15	10.9
Cytoplasmic					
PEST	Pest	84	71.2	73	65.5
1B	1B	15	12.7	14	12.7
BAND 4.1	Meg	2	1.7	1	0.9
	D1	2	1.7	0	0

^aThe observed incidences are given as absolute numbers (n) and as a percentage of all PTP clones derived from that cell type. It should be emphasized, however, that this gives little information on the original relative abundance of transcripts as other factors contribute to the frequency of amplification by PCR.

Immunoblotting analysis Cells were washed in phosphate-buffered saline and lysed in RIPA buffer containing protease inhibitors; biopsies were processed for electrophoresis as previously described (Easty *et al.*, 1995a). The protein concentration of each sample was determined using the BCA protein assay (Pierce, Rockford, IL). Samples were electrophoresed through 6% gels and transferred to nitrocellulose membranes (Pall Corporation, MI). Immunoblotting was carried out using an affinity purified goat polyclonal specific antibody for PTP-κ (Santa Cruz Biotechnology, Santa Cruz, CA), diluted 1:1000. Binding of the primary antibody was detected using a Vectorstain ABC kit; membranes were incubated with biotin conjugated antigoat antibody (diluted 1:2000), followed by peroxidase conjugated avidin according to the manufacturer's instructions (Vector, Burlingame, CA). Specific antibody binding was determined using an enhanced chemoluminescence detection kit (ECL, Amersham, Arlington Heights, IL), as per the manufacturer's instructions.

RESULTS

PCR cloning of PTP from melanocytes and melanoma cells

We used highly degenerate primers directed to the conserved catalytic domain for RT-PCR to isolate PTP expressed in melanocytes and a melanoma cell line (451LU). The amplified products (approximately 350–400 bp) were cloned and sequenced. For each cell type approximately 200 clones were isolated, and 118 (from melanocytes) and 113 (from 451LU) had sequence identity, or homology to known PTP when compared to EMBL and GenBank databases. In total, nine different (five receptor and four cytoplasmic) partial PTP genes were identified (Table I). Seven PTP were isolated from 451LU cells. Out of these, PTP-γ was not found in normal melanocytes. On the other hand, eight distinct PTP were present in melanocytes, and two of these (PTP-π and PTP-D1) were not isolated from 451LU cells. The incidence of PTP-PEST clones was high in both normal melanocytes and malignant melanoma cells and may reflect abundant expression of this gene, seen by northern blotting analysis. The numbers of clones isolated corresponding to each specific PTP gene are likely to be determined by the primer sequences used for PCR and the absolute amount of mRNA present for each PTP gene. The incidence is nonquantitative, but provides an indication of PTP gene expression in each cell type. In particular, as only low numbers of clones were isolated for PTP-π and PTP-D1 from the normal melanocytes, we could not discount the possibility that melanoma cell line 451LU might also express both of these genes.

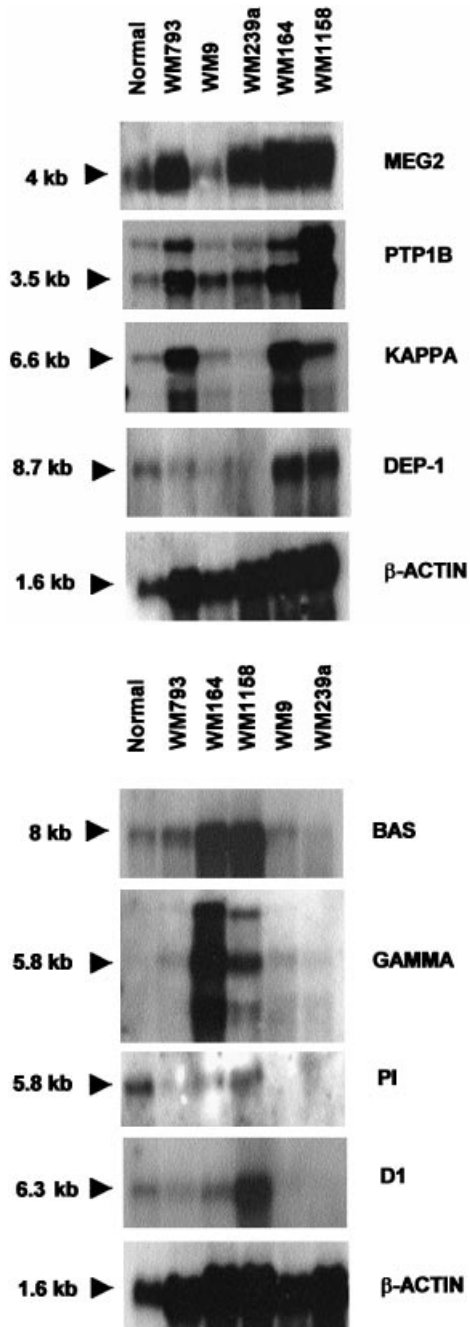


Figure 1. Northern blot analysis of poly(A⁺) RNA enriched from normal melanocytes and melanoma cell lines (5 μg per lane). The normal melanocyte RNA is under-loaded. (a) Hybridization was to MEG2, PTP-1B, PTP-κ, DEP-1, and β-ACTIN probes; durations of exposure were 7, 3, 2, 7, and 1 d, respectively. (b) Hybridization was to PTP-BAS, PTP-γ, PTP-π, PTP-D1, and β-ACTIN probes; durations of exposure were 2, 2, 12, 2, and 1 d, respectively.

Northern blotting analysis of PTP Northern blotting analysis was used to investigate the expression of PTP isolated by RT-PCR. A panel of melanocytes and melanoma cell lines was tested to determine expression of 15 PTP genes, and representatives of these blots are shown in Fig 1(a, b); Table II contains data from laser densitometry. Only the PTP-MEG1 probe failed to detect a message; signals for all of the remaining genes were found in some cultured melanocytic cells. The expression of PTP differed between normal melanocytes and melanoma cell lines. Two cytoplasmic (PTP-1B and PTP-H1) and one receptor (PTP-γ) sequences were

Table II. Quantitation of PTP mRNA expression in human melanocyte and melanoma cell lines

	Phosphatase			
	MEG2	1B	κ	DEP-1
Experiment 1 ^b				
Normal 2	100	100	100	100
Wm793	104	505	363	7
WM9	7	219	31	Trc.
WM239a	104	265	Trc.	Trc.
Wm1158	141	792	395	76
Wm164	122	986	173	65
	BAS	γ	D1	PI
Experiment 2 ^b				
Normal 2	100	Trc.	100	100
WM793	53	100	8	Trc.
Wm164	163	1876	224	ND
Wm1158	189	887	887	ND
WM9	31	145	Trc.	0
WM239a	Trc.	Trc.	Trc.	Trc.
Cells ^c	π	α		
Experiment 3 ^b				
Normal 1	100	100		
Wm793	30	172		
Wm852	20	236		
Wm9	0	303		
Wm164	22	180		
Dx3	361	63		
Wm1158	34	164		
Wm983a	0	157		
Experiment 4 ^b				
PTP- κ was detected in three cell lines derived from metastatic melanoma (DX3, COLO832, A375P) and four cell lines (WM1650, WM793, WM35, and WM1341B) derived from early melanomas; WM1341B cells (and sublines derived from WM1341B) contained trace amounts of PTP- κ .				

^aAll cell lines are metastatic melanomas except the Radial Growth Phase (non-metastatic) cell line WM793 and the melanocytes (Normal).

^bFor experiment 1, levels of expression were normalized to Normal 2 (defined as 100). For experiment 2 levels of expression were normalized to Normal 2; except for PTP- γ where expression was compared with WM793. Experiment 3 was normalized to Normal 1. Trc. indicates trace amounts of RNA are present that could not be quantitated based upon laser densitometry; 0 indicates that no signal was detectable. Expression was regarded as significantly different when there was 3-fold variation compared to the reference cell line for two or more melanoma cell lines. ND (not done); for the PTP- π blot the high background compromised densitometry and these values are not included here.

^cPTP mRNA expression was quantitated by laser densitometry of autoradiographs. Relative expression levels were normalized to GAPDH mRNA expression and to one cell line for each probe as described (Materials and Methods). Data are from four independent experiments as shown.

more highly expressed in some melanomas (Fig 1, and data not shown). This is consistent with the absence of PTP- γ from clones isolated from normal melanocytes by RT-PCR (Table I). On the other hand, several PTP genes (PTP- κ , PTP- π , PTP-BAS, and PTP-D1) showed loss or downregulated expression in two or more malignant melanoma cell lines. The normal melanocyte samples in Fig 1 are under-loaded; however, this does not affect the conclusion that PTP expression was decreased in some melanoma cell lines. Whereas a signal for PTP- κ was clearly visible in (the under-loaded) normal melanocytes, WM9 contained only trace amounts of PTP- κ , and no transcript was detected in WM239a cells (Fig 1a). Similarly, PTP- π was found in normal melanocytes (Fig 1b) but was not detected in WM793 and WM239a cells. PTP-D1 was expressed in four out of six melanoma cell lines in quantities greater than trace amounts; in one cell line (WM164), expression of PTP-D1 was increased compared to melanocytes.

In an initial blot, expression of PTP- κ was increased in two melanoma cell lines (Fig 1a); however, when PTP gene expression was tested on a larger panel of cell lines PTP- κ and PTP- π were consistently downregulated in multiple samples (Fig 2a, b). These

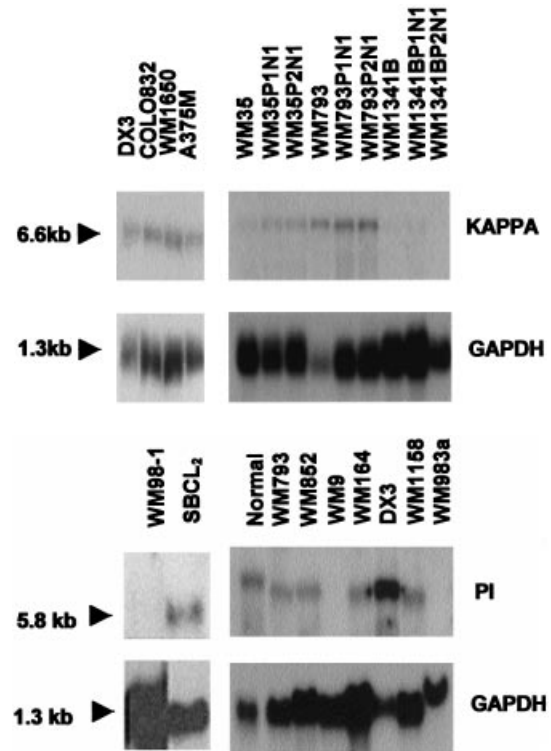


Figure 2. Northern blot analysis of poly(A⁺)-enriched RNA from normal melanocytes and melanoma cell lines (5 μ g per lane). (a) Hybridization was to PTP- κ and GAPDH probes; durations of exposure were 1 d and 1 h, respectively. (b) Hybridization was to PTP- π and GAPDH probes; durations of exposure were 4 d and 1 h, respectively.

two closely related members of a small family of receptors, previously implicated in the mediation of contact inhibition (Fuchs *et al*, 1996), were chosen for further study. PTP- κ was detected in three of three normal melanocyte cultures. Only 11 of 14 (78%) melanoma cell lines contained more than trace amounts of PTP- κ message, however (Table III); with extended autoradiography a signal was detected for WM1341B (Fig 2a). Matrigel selection did not significantly affect PTP- κ expression and derived lines (P1N1, P1N2) generally maintained similar expression patterns to the parental cell line. In Table III each group of parental melanoma and derived subclones was scored as a single observation. Interestingly, PTP- κ was detected in three of four cell lines derived from early primary melanomas suggesting that loss of expression may occur early in progression in some melanomas (Table III). PTP- π was found in two of two normal melanocytes and in SBC12 cells derived from an early melanoma, but was not detected in three of ten (30%) melanoma cell lines (Fig 2b); in addition, expression was downregulated in most (five of nine) of the melanoma cell lines tested. In some cases both PTP- κ and PTP- π were downregulated in the same cell line (including WM239A).

Unmanipulated melanoma biopsies were tested by northern blotting analysis; representative blots are shown in Fig 3. PTP-BAS was detected in RNA derived from six of nine metastatic melanoma biopsies. In the case of PTP- κ , 13 of 25 samples were strongly positive; a further four biopsies contained small amounts of message, and in eight of 25 biopsies no signal was detected. PTP- π was easily detected in only one of nine melanoma biopsies; trace amounts were found in three further samples, and six of nine biopsies did not contain PTP- π message. Interestingly, the expression of PTP- π and PTP- κ overlapped to some degree; both genes were lost in some melanoma biopsies and four of six of the biopsies positive for PTP- κ also contained PTP- π . Expression

Table III. Comparison of PTP- κ and PTP- π mRNA expression in cell lines from various stages of melanoma progression

mRNA	Melanocytes	Primary melanoma	Metastatic melanoma	Uncultured metastases
κ	3/3 ^a	3/4	9/11	17/25
π	2/2	3/3	5/7	4/9

^aExpression of selected mRNAs is shown as number of cell lines containing mRNA in quantities greater than trace amounts divided by the number of samples tested. Uncultured metastases were biopsy specimens (see *Materials and Methods*). Expression was quantitated by laser densitometry of autoradiographs; for cultured cells relative expression was normalized to that in melanocytes.

did not appear to correlate with histopathology of the primary melanoma or clinical data (survival time) of the patient.

Southern blotting analysis of PTP To test for alterations in PTP genes, control lymphoblastoid cells were compared with melanoma cell lines by southern blotting analysis; we found no evidence for deletions or gross rearrangements of PTP- κ or PTP- π .

Immunoblotting analysis A specific polyclonal antibody was used to determine expression of PTP- κ in melanocytic cells; an antibody for PTP- π was not available. Cell lysates from pigmented cells and positive controls gave immunoreaction signals that were barely above background. These did not yield acceptable data for quantitation and are not included here. Hence the antikappa antibody did not provide consistent results. To the author's knowledge this is the only antikappa antibody currently commercially available.

DISCUSSION

Melanomas and the cell lines derived from them contain increased amounts of phospho-tyrosine compared to their normal cellular counterparts. This appears to reflect overexpression of PTK, enzymes encoded by a multigene family, which includes transforming oncogenes. On the other hand, loss of PTP expression may also contribute to increased phospho-tyrosine. Our hypothesis was that PTP, downregulated or lost during melanoma progression, would include the products of tumor suppressor genes. In order to learn more about PTP expression, we used RT-PCR to isolate partial cDNAs from normal and malignant melanocytes.

Specific PTP sequences detected in pigment cells We have identified 15 different PTP, and significantly extended the repertoire of known PTP in melanocytic cells. A number of phosphatases were identified here only once and others present were probably not found by our PCR analysis. For example, PTP-DELTA and PTP-ZETA expression have been detected in melanoma cells using other methods (Halaban *et al.*, 1992; Goldmann *et al.*, 2000). Some omissions are expected, however, as the products of PCR are influenced by stochastic events, target sequence, and concentration. The role of PTP in human melanoma has received little attention; to date there has only been one previous study. Halaban *et al.* (1992) found seven PTP present in both melanoma cell lines and normal melanocytes; PTP-DELTA was not detected in normal melanocytes, but was present in two out of four melanoma cell lines.

We find a complex pattern of expression for 14 PTP genes; specific increases and decreases in PTP expression occurred in malignant cells. PTP-1B, PTP-H1, PTP-DEP, and PTP- γ were overexpressed in some melanoma cell lines. Increased expression of PTP genes has previously been found in other malignant cells, including breast cancer cell lines (Zhai *et al.*, 1993). The reason for increased PTP expression in cancer cells is unclear, but activation of oncogenes (such as ERB-B2 in breast cancer) may result in phosphorylation of multiple substrates, including those involved in

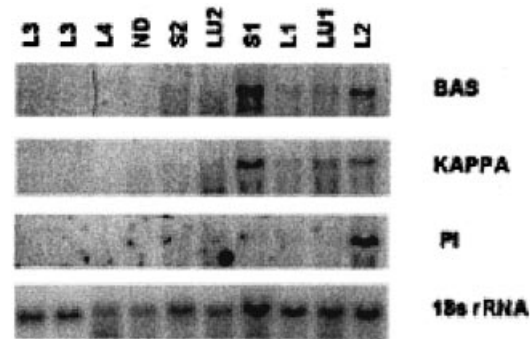


Figure 3. Northern blot analysis of total RNA from human metastatic melanoma biopsies (5 μ g per lane). L, S, Lu, samples derived from lymph node, subcutaneous and lung metastases, respectively; ND, site of metastasis not determined. Hybridization was to PTP-BAS, PTP- κ , and PTP- π probes; an 18S rRNA specific oligonucleotide was used for quantitation. Durations of exposure were 5, 8, 12, and 1 d, respectively.

growth and morphologic transformation, but also housekeeping proteins involved in other essential metabolic processes. Hence, PTK overexpression may be deleterious and this effect may be circumvented by increases in the expression of specific PTP.

It would also be of interest to study PTP in melanoma at the protein level. Due to the lack of presently available suitable antibodies specific for PTP, however, this is not currently possible. This present situation perhaps reflects the paucity of previous studies concerning PTP in comparison to PTK, and suggests that PTP have been neglected and will be an important area for further research.

PTP- κ and PTP- π Two PTP genes were selected for further study: PTP- κ and PTP- π . Expression of these genes was decreased or lost compared to normal melanocytes in more than 20% of melanoma cell lines. The relevance of results from cultured cells to the potential expression and function in unmanipulated tumors was unclear, however. We therefore studied gene expression of PTP- κ and PTP- π in unmanipulated biopsies and again found this was reduced below levels required for detection in multiple melanoma samples.

Several studies have shown that receptor PTP are involved in cell-cell adhesion (Neel and Tonks, 1997). PTP- κ and PTP- π belong to the small 2B subgroup of receptor PTP that includes PTP-MU. These receptors consist of an extracellular segment containing a MAM domain, an Ig domain, four fibronectin-type III repeats, a transmembrane segment, and two tandem intracellular PTP domains (Neel and Tonks, 1997). The role of the 2B family of receptor PTP is uncertain and experiments are currently under way to determine the effect of forced expression of PTP- κ in melanoma cells that do not express this protein. As PTP- κ and PTP-MU are localized to adherens junctions, mediate homotypic adhesion *in vitro*, and regulate phosphorylation of cadherin-catenin complexes (Brady-Kalnay *et al.*, 1998), they may contribute to cell-cell communication (Fuchs *et al.*, 1996).

PTP- κ seems to be a particularly good candidate as a tumor suppressor gene; it is upregulated by transforming growth factor β (a negative regulator of cell growth) and has been implicated in contact inhibition (Yang *et al.*, 1996). In addition, PTP- κ has been mapped to 6q22.2-3 (Zhang *et al.*, 1998), a common site for chromosome rearrangements in melanoma (Albino and Fountain, 1993). The close sequence homology of these downregulated genes suggests that the proteins they encode will have similar cellular functions. In this regard, it is not clear whether downregulation of both gene products (PTP- κ and PTP- π) is required for melanoma genesis; interestingly, this did occur in some of the melanoma cell lines and biopsies tested. We found loss of or decreased expression of PTP- κ in some cell lines derived from early melanomas; in

contrast, overexpression of PTK (including EPH-A2; Easty *et al*, 1995a; Bittner *et al*, 2000) has generally been reported in advanced melanomas (Easty and Bennett, 2000). One possible explanation may be that loss of PTP(s) occurs as an early event, and that subsequent small changes in PTK(s) expression are late events in melanoma progression. This sort of interaction (between PTK and PTP) has been hypothesized to occur in other human tumors. In chronic myeloid leukemia cells, recent data suggest that, in addition to generation of Bcr-*abl*, loss of a PTP is necessary for transformation (LaMontagne *et al*, 1998). Moreover, aggressive human breast cancer cell lines (rapid growing in athymic mice) contained high expression of ERB-B2 and lower levels of PTP compared to slower growing tumors (Zhai *et al*, 1993).

In summary, we report abnormal PTP expression in well-characterized melanoma cell lines previously shown to overexpress specific PTK (Easty and Bennett, 2000). As the activity of PTP is significantly greater than that of PTK (Neel and Tonks, 1997), increases in PTK expression appear more likely to be functionally significant when combined with downregulation of PTP activity. Taken together, these changes offer an explanation for the abnormal phospho-tyrosine previously reported in melanoma. Upregulation of PTK expression has been implicated in melanoma genesis; it seems likely that decreased PTP activity will also impact upon the control of this process.

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