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Gene expression in melanoma cell lines and cultured melanocytes: correlation between levels of c-*src*-1, c-*myc* and p53

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The molecular genetics of melanoma is little understood and has concentrated largely on DNA analyses. We have examined mRNA levels of 21 different oncogenes, anti-oncogenes, growth factors and proteases in cultured melanocytes and 17 melanoma cell lines. C-*mel*, c-*erb*-B2, c-*myc*, c-*src*-1, p53, platelet derived growth factor A chain, *gro*, transforming growth factor α, epidermal growth factor receptor and tissue plasminogen activator were all expressed in at least some cell lines. Most striking was the finding that there are significant inter-correlations of c-*myc*, p53 and c-*src*-1 levels, and between p53 and c-*erb*-B2, which may be due to common regulatory control of these genes in cells of the melanocytic lineage.

Introduction

Tumorigenesis is thought to occur as a result of genetic changes which lead to both initiation and promotion. In retinoblastoma, the primary gene involved has been identified (Lee *et al.*, 1987) but in most tumours a variety of genetic changes has been found, the significance of which for initiation, promotion, maintenance and metastasis is unknown. The genes likely to be involved in these steps include proto-oncogenes, anti-oncogenes, growth factors and growth factor receptors. In addition, the behaviour of the resulting tumour may be determined in part by the expression of proteases such as plasminogen activators. Since most of these genes may be expressed in a tissue-specific manner, reflecting their normal role in differentiation, exactly which ones are involved in tumorigenesis will depend on the type of tumour.

The molecular genetics of malignant melanoma is little understood and most work has concentrated on DNA analyses. Activated c-Ha-*ras* and c-N-*ras* oncogenes have been detected in about 12% of metastatic melanoma cell lines with the NIH3T3 transfection assay (Albino, 1988), but not in lines derived from primary tissue. This suggests that activation of these genes might be related to metastasis. However, more recently, van't Veer *et al.* (1989) used a combination of the polymerase chain reaction and oligonucleotide hybridization and found N-*ras* mutations in 19% of patients with melanoma. Most importantly, in all cases where there was primary tissue as well as metastatic material available, the mutation was present in both. All the primary tumours with activated N-*ras* genes occurred in areas which had been exposed to sunlight, suggesting an active role for ultraviolet light in their induction. Padua *et al.* (1984) identified a novel oncogene, c-*mel*, by transfection experiments in one melanoma cell line, but no generalized role for this gene in melanoma has been established.

There has also been interest in the possibility that 'anti-oncogenes' or suppressor genes play a role in melanoma, as in retinoblastoma (Lee *et al.*, 1987), and that recessive mutations in such genes might be uncovered by loss of the normal allele. Dracopoli *et al.* (1985) used RFLPs to detect allele loss on 11 chromosomes in melanoma cell lines and concluded that such loss is common but not restricted to a particular locus (which might indicate the presence of a key suppressor gene). In six pedigrees multiplex for melanoma, a single 'melanoma gene' has been claimed to be mapped to chromosome 1 by linkage analysis in some familial melanoma pedigrees (Bale *et al.*, 1989). However, this finding has not been confirmed by others (van Haeringen *et al.*, 1989), and the nature of the gene and its product awaits its isolation. Further work by Dracopoli *et al.* (1989) suggests that loss of heterozygosity at 1p is a common finding in melanoma, but that it occurs late in tumorigenesis during progression. The relationship between these data and the mapping of the 'familial melanoma gene' is not clear.

Another approach to finding predisposing genes has been to look for associations of melanoma with rare alleles of candidate genes. The results here are contradictory, with some groups finding an association between rare c-Ha-*ras* alleles and melanoma (Hayward *et al.*, 1988) and others finding no such association (e.g. Gerhard *et al.*, 1987). There have been two major studies aimed at detecting rearrangement and amplification of specific oncogenes in melanomas (Linnenbach *et al.*, 1988; Albino, 1988). One cell line derived from a primary melanoma was found to have a c-*myb* rearrangement. In addition, 10–15% of cultured melanomas had minimal (1.5–3 fold) c-*myc* amplification which may reflect changes that occurred *in vitro* at either the chromosomal or gene level. In summary, it is likely that the lack of a common molecular abnormality and the divergent results of these approaches reflect (a) the heterogeneity of malignant melanomas and (b) the multistep of carcinogenesis.

In melanoma, as in other malignancies, proto-oncogenes can be activated by rearrangement, point mutation or amplification. It is likely that similar mechanisms of activation exist for all the classes of genes thought to be involved in transformation. Each of these types of mutation can affect levels of gene expression and the size of the transcripts (although this is not necessarily so). There have, however, been few studies of gene expression in melanoma aimed at detecting gene

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activation in this way. In one report, a range of melanoma cell lines representing three different levels of differentiation, with cultured melanocytes as a control, was examined for expression of c-*myc*, N-*myc*, c-Ha-*ras*, c-K-*ras*, N-*ras*, c-*src*-1, c-*fos* and c-*sis* (Albino, 1988). With the exception of c-*sis* (which codes for the B-chain of platelet derived growth factor (PDGF)), all these genes were expressed, but at the same level and with the same-sized transcript as in the normal melanocytes. Ogiso *et al.* (1988) examined the expression of c-Ha-*ras*, N-*ras*, c-*fos*, c-*myc* and the epidermal growth factor receptor (EGF-R) in four un-cultured melanomas compared with normal epidermis and cellular nevi. EGF-R was not expressed in any of the melanomas, but all the other genes examined were expressed in a manner that was both quantitatively and qualitatively similar to the controls.

In an attempt to discover which genes might be active in melanomas, we have examined mRNA levels of 21 different oncogenes, growth factors, growth factor receptors and proteases in cultured melanocytes and 17 malignant melanoma cell lines. Since we found considerable variation in the levels of expression between the cell lines, we hypothesized that co-ordinated expression of subsets of genes might be reflected in significant correlations between their mRNA levels across lines. Indeed, we found significant correlations between c-*myc*, c-*src*-1, p53 and c-*erb*-B2 levels.

Results

No mRNA was detected by Northern analysis for urokinase plasminogen activator (u-PA), basic fibroblast growth factor (bFGF), epidermal growth factor (EGF), platelet derived growth factor (PDGF) receptor, PDGF-B/c-*sis*, c-*int*2, c-*yes*, c-*hst*, c-*dbl*, L-*myc* or N-*myc*. At least 7 μg polyA$^+$ RNA was loaded from each sample and often separate blots were made with 15 μg, but even they were negative with these probes.

In contrast, c-*mel*, c-*erb*-B2, c-*myc*, c-*src*-1, p53, PDGF-A, *gro*, transforming growth factor alpha (TGFα), EGF receptor and tissue plasminogen activator (t-PA) were expressed at varying levels in at least some cell lines (Table 1; Figure 1). The intensity of the signal was quantified for all these genes except c-*mel*, EGF-R and PDGF-A for which the signal was too low for meaningful densitometry (and hence inclusion in the correlation analyses). The results of the densitometry cannot be used to compare levels of different mRNAs within a cell line (since they were obtained from different blots) but since, for a given probe, all 18 cell lines are measured on the same blot we can examine correlations in the expression of different genes over all the cell lines. Overall levels of expression based on length of exposure and signal strength, which can be used to compare expression of different genes within a line, are given in Table 1. No aberrant molecular weight species were identified and, with the exception of c-*mel*, the message sizes were consistent with published reports. The only published report of c-*mel* expression, to our knowledge, found a 3.5 kb mRNA whereas the message we detected was only 1.4 kb (Padua *et al.*, 1985). In addition to the main TGFα at 4.1 kb, in some of our cell lines we also detected lower levels of 10.5 kb and/or 1.5 kb transcripts.

Since the MM229 cell line had exceptionally high levels of t-PA, TGFα and *gro* expression, Southern analyses were performed with EcoRI, BamHI and

Table 1 Expression levels in melanocytes and melanoma cell lines

Cells	*Source*	*c-src-1*	*c-myc*	*p53*	*c-erb-B2*	*gro/MGSA*	*TGFα*	*t-PA*	*c-mel*	*PDGF-A*	*EGF-R*
Melanocytes		0.14	0.00	ND**	ND	0.83	0.00	0.00	+	0.00	0.00
MM200	Primary	2.38	3.89	2.84	0.37	3.14	0.00	1.65	+	0.00	0.00
MM418	melanomas	0.10	0.85	3.49	3.15	0.00	0.00	0.02	+	+	+
COL0239F		ND	0.00	ND	ND	15.32	0.00	0.06	ND	ND	0.00
MM96		0.01	0.06	0.30	0.66	1.77	0.24L	0.00	+	0.00	0.00
MM127	M	0.36	2.86	2.47	0.20	0.00	0.01	10.27	+	0.00	+
MM138	E	3.23	4.99	4.40	3.06	1.07	0.00	0.00	+	0.00	0.00
MM170	T	1.27	1.56	1.59	0.11	0.00	1.06^L	0.01	+	+	+
MM229	A	0.08	0.19	1.93	3.07	40.32	30.70^{HL}	17.72	+	+	0.00
MM409	S	0.28	0.95	0.78	1.20	0.21	0.02	0.29	+	0.00	0.00
MM455	T	0.00	0.00	0.56	0.12	0.00	0.89^L	0.01	+	+	0.00
MM472	A	0.20	0.01	1.22	0.10	0.31	1.00^L	0.00	+	0.00	0.00
MM473	T	1.56	10.33	2.71	1.22	2.17	0.00	0.00	+	0.00	0.00
MM485	I	0.58	1.56	0.33	0.06	0.00	0.72^L	0.47	+	+	0.00
BL	C	0.04	1.18	1.47	0.12	0.01	0.80	2.26	+	+	0.00
NKI-4		0.01	0.43	0.57	0.03	0.00	0.06	0.16	+	0.00	0.00
Me113	?	0.09	0.17	1.86	1.20	0.00	0.00	0.03	+	0.00	0.00
WW	?	0.01	1.52	0.79	0.03	2.84	0.69	0.55	+	0.00	0.00
Molecular weights (kb)		4.1	2.3	2.3	4.1	1.4	4.3	2.4	1.4	2.2	9.5, 5.5
Overall level of expression*		nil–moderate	nil–low	nil–moderate	low	nil–high	nil–high	nil–high	v. low	v. low	v. low

Expression levels are estimated by the ratio of the intensity of the hybridization signal for the probe of interest to that for GAPDH
* Ratios are not comparable between genes but overall levels of expression are given based on length of exposure and mean signal strength
+ : signal was too low for meaningful densitometry
L: also a 1.2 kb message
H: also a 10.5 kb message
ND: not determined
ND**: not determined on this blot, but positive on another

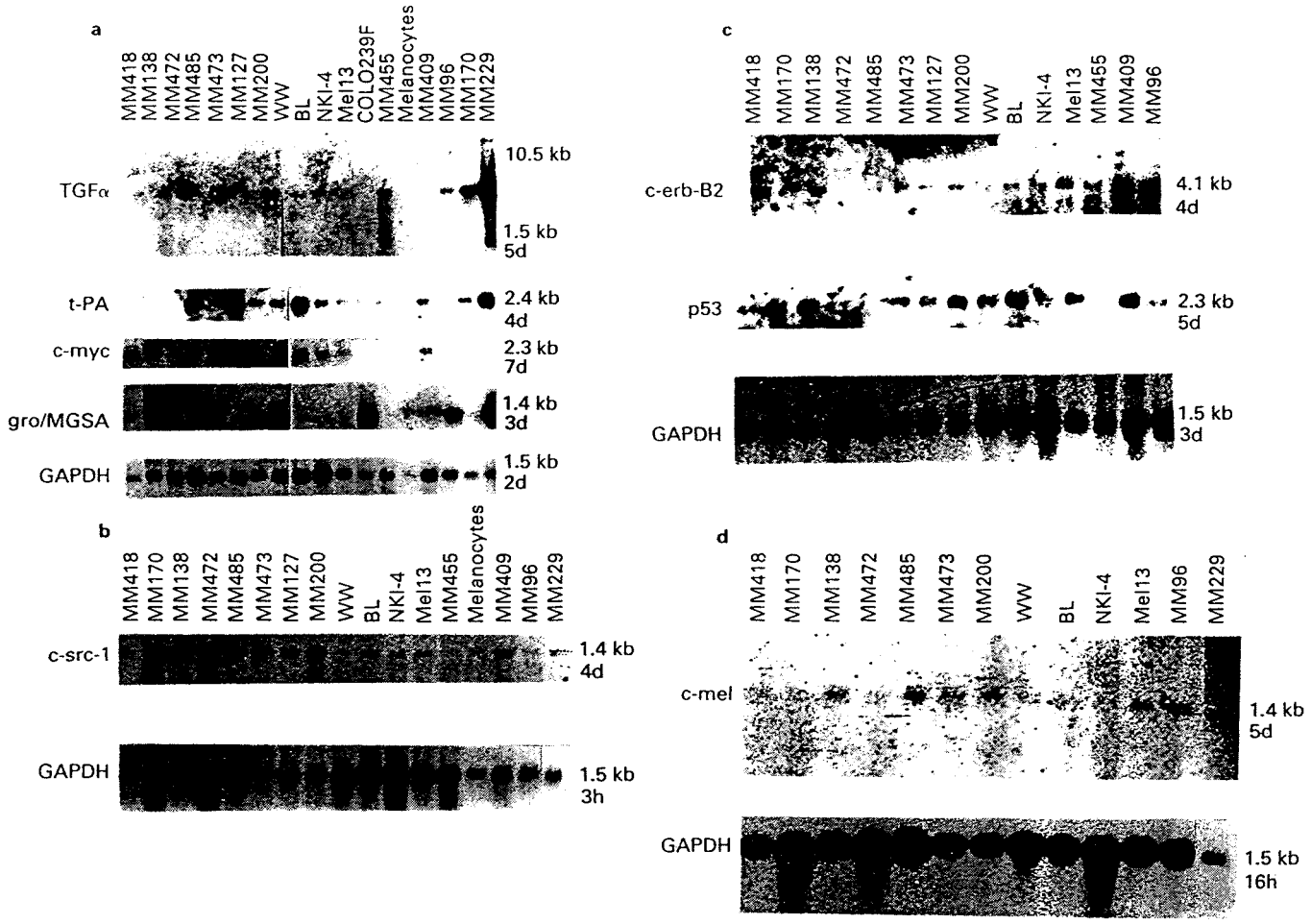

Figure 1 Northern analyses in melanocytes and melanoma cell lines. 7 μg poly-A$^+$ RNA was fractionated on formaldehyde gels and transfered to charged nylon membranes. Filters were hybridized to nick-translated probes and washed at high stringency. Molecular weights of the transcripts and exposure time of the autoradiographs are given

HindIII digests to examine the gross structure of these genes in this cell line. No rearrangements were detected (data not shown) but of course this does not rule out small mutations affecting transcription levels.

All pair-wise correlations for levels of mRNA were calculated between c-*src*-1, c-*myc*, p53, *gro*, t-PA, TGFα and c-*erb*-B2 (Table 2). There are very high inter-correlations of *gro*, t-PA and TGFα, but inspection of the data revealed that these are entirely due to very high expression of these three genes in just one cell line, MM229. Similarly, the correlations of c-*erb*-B2 with *gro* and TGFα were due largely to the high expression levels in MM229.

In contrast, the correlations between p53 and c-*erb*-B2, and the intercorrelations of c-*myc*, c-*src*-1 and p53 do not arise from extreme results in any one cell line but appear to reflect a proportionality of expression across all cell lines (Figure 2). The levels of expression of c-*myc*, c-*src*-1 and p53 were taken from three different Northern blots with three different sets of GAPDH control measurements which makes it unlikely that the correlations are an artifact resulting from a compounding error in densitometry. The correlation matrix was subjected to principal components analysis, and two factors were extracted and subjected to varimax rotation (SPSS, 1988). The first factor merely reflects the high expression of *gro*, TGFα, t-PA and c-*erb*-B2 in line MM229. The second factor had loadings of 0.88, 0.79 and 0.90 on *src*, *myc* and p53 respectively, with a smaller loading of 0.55 on *erb*-B2. This implies that a

Table 2 Correlation matrix of expression levels

	c-src-1	*gro/MGSA*	*TGFα*	*t-PA*	*c-myc*	*p53*
gro/MGSA	−0.16					
TGFα	−0.10	0.93***				
t-PA	−0.15	0.77***	0.85***			
c-myc	0.68**	−0.15	−0.16	−0.06		
p53	0.69**	0.07	0.02	0.13	0.55*	
c-erb-B2	0.27	0.49+	0.47+	0.32	0.15	0.67*

Two tail tests of significance:
+ $0.05 < P < 0.1$
* $0.01 < P < 0.05$
** $0.001 < P < 0.01$
*** $P < 0.001$

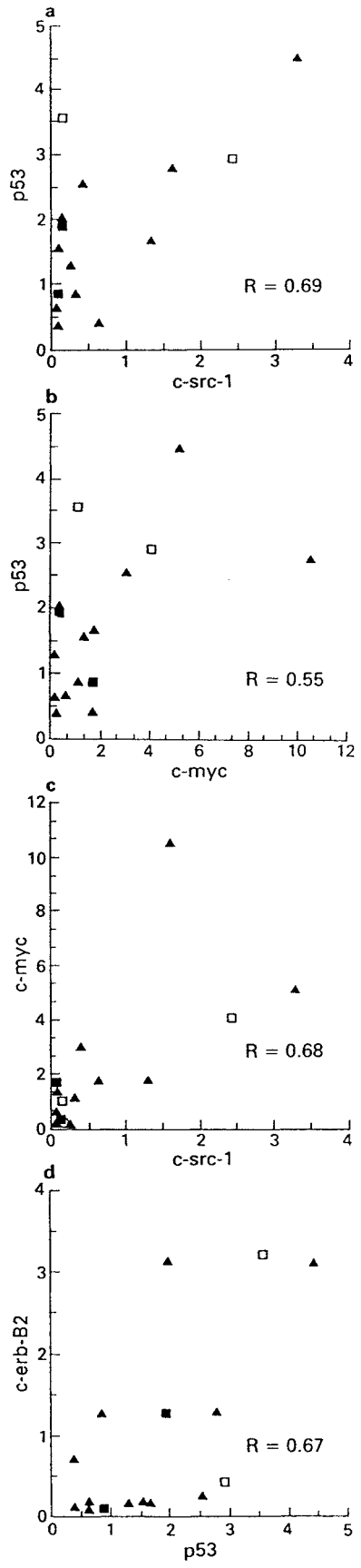

Figure 2 Scattergraphs of gene expression in melanocytes and melanoma cell lines. ○ Melanocytes, □ Primary cell line, ▲ Metastatic cell line, ■ Unknown

common regulatory factor accounts for 77%, 62% and 81% of variance between lines in *src*, *myc* and p53, and also 30% of the variance in *erb*-B2. Given the errors in measurement inherent in densitometric estimation of gene expression, this points to a very high level of joint control of expression of *myc*, *src* and p53, and to some extent *erb*-B2, in melanoma cells.

Discussion

Growth factors and growth factor receptors

Several lines of evidence, including its stimulation by ultraviolet light (Ellem *et al.*, 1988), indicate that TGFα plays an important role in melanoma. The major transcript is reported to be 4.3–4.8 kb, but smaller species of unknown function have also been detected (Coffey *et al.*, 1987; Imanishi *et al.*, 1989). We can detect TGFα mRNA, at varying levels, in 11/17 of our cell lines but not in normal melanocytes. In addition to the 4.3 and 1.5 kb transcripts, the MM229 line also has a 10.5 kb message (Figure 1a). The mode of action of TGFα is uncertain since only 3/17 of our cell lines produce detectable mRNA for the EGF-R (the natural receptor for TGFα). This makes a classical autocrine mechanism less likely in these cells although we are aware that this conclusion assumes a good correlation between mRNA and protein levels which, for TGFα, we know to be untrue for our cell lines (Ellem *et al.*, in preparation).

The lack of detectable mRNA for bFGF in any of our cell lines is surprising since bFGF is reported to be the natural mitogen for melanocytes and to act in an autocrine manner in melanoma (Halaban, 1988; Halaban *et al.*, 1988a,b). It is normally produced by keratinocytes, but not by normal melanocytes nor melanocytes derived from primary melanomas. In contrast, Halaban *et al.* (1988b) report that bFGF mRNA is detected in, and their data indicate that it is produced by, metastatic melanoma cells, thereby rendering them independent of an external source for proliferation. Halaban *et al.* used a bovine cDNA probe while we used a human cDNA probe which if anything should improve sensitivity. Since most of our cell lines are derived from metastatic melanomas, we can only suggest that if they are producing bFGF it is from very low levels of mRNA. We also failed to detect mRNA for two oncogenes which are putative members of the bFGF gene family—*hst* and *int*-2 (Yoshida *et al.*, 1987). This makes it unlikely that they play an important role in the proliferation of melanoma cells despite a report of their co-amplification in a human melanoma (Adelaide *et al.*, 1988).

In contrast, we detected mRNA for the growth factor, *gro*, in most of our cell lines and in normal melanocytes. *Gro* is identical to melanocyte stimulatory activity (MGSA) which may act in an autocrine manner in melanoma cells and can induce its own synthesis (Richmond *et al.*, 1988). Its transcription has been previously reported in melanoma cells but not, to our knowledge, in normal melanocytes. Since we can detect *gro* mRNA in normal melanocytes, but not in all of our malignant melanoma cell lines it is unlikely that activation of this gene *per se* is responsible for the transformation. We find widely varying levels of *gro* message in our cells and it would be interesting to know whether this is reflected at the protein level.

Westermark *et al.* (1986) detected PDGF activity in 3/5 melanoma cell lines (all of which originated from

the same patient). The activity was ascribed to an A-chain homodimer but transcription of the B-chain was also found in some melanoma cell lines. We detected low levels of PDGF-A chain mRNA in 8/16 melanoma cell lines but not in normal melanocytes. No PDGF-B chain (c-*sis*) nor PDGF receptor mRNA was detected by our Northern analyses. Whether a functional A-chain homodimer is produced by these cells, and what its role is in melanoma is unknown.

Oncogenes

The only member of the myc family of oncogenes found to be expressed in this study was c-*myc*. This is in contrast to Albino (1988) who also detected N-*myc* mRNA in melanoma cells. C-*myc* mRNA was detected at a low level in 16/18 melanoma cell lines, but not in normal melanocytes. (However it should be noted that the apparent absence in normal melanocytes might be because, as indicated by the GAPDH level, less mRNA was loaded onto the gel). The function of this family of nuclear oncogenes is unclear but they are found to be expressed in a tissue specific manner and c-*myc* is known to be expressed most strongly in proliferating tissues (for review see DePinho *et al.*, 1987). Variation in proliferation rate among our cells might explain the absence of detectable c-*myc* mRNA in melanocytes and COLO239F and MM455 melanoma cells although all cells were harvested from confluent plates.

No transcription was detected for the oncogenes c-*dbl* and c-*yes*. However, we report for the first time in melanoma cells, transcription of c-*erb*-B2 (or c-*neu*) which codes for a growth factor receptor which is homologous to EGF-R but for which the ligand is unknown. With the exception of MM455 melanoma cells, c-*src*-1 was expressed in all cell lines tested, and in normal melanocytes. C-*src*-1 codes for the membrane-bound tyrosine kinase, $pp60^{src}$ and there is some evidence that it can control cellular growth by regulating cell-to-cell communication (Azarnia *et al.*, 1988). C-*mel* was expressed at a very low level in all the cells we examined (including the NKI-4 line from which it was originally isolated). Surprisingly, there have not been any reports of the expression of this oncogene in cells of the meanlocytic lineage, despite its association with melanoma. The transcript in our cells was approximately 1.4 kb; that reported by Padua *et al.* (1984) in an unspecified tissue, was 3.5 kb.

Anti-oncogenes

The gene for the tumour specific antigen, p53, was expressed in all melanoma cell lines tested, and in normal melanocytes. There is increasing evidence that this nuclear protein functions as a suppressor of tumorigenesis and can be inactivated by certain mutations, making the cell susceptible to uncontrolled proliferation (Finlay *et al.*, 1989). Whether the p53 gene produces a normal, suppressing p53 protein, or an inactive form, in melanoma cells is not known.

Plasminogen activators

Plasminogen activators convert inactive plasminogen to the active protease, plasmin, and as such have been implicated in metastasis. No u-PA mRNA was detected in these melanocytic cells, which is consistent with previous reports (Rijken & Collen, 1981), but varying levels of t-PA mRNA were detected in 13/17 cell lines. No t-PA expression was detectable in normal melanocytes, but all four cell lines without t-PA expression were derived from metastases so there is no simple association between t-PA transcription and metastasis in these cell lines.

Relative gene expression in melanocytes versus melanoma cell lines, both primary and metastatic

One of the purposes of this study was to compare gene expression in melanocytes versus melanoma cells, and in melanoma cell lines derived from primary tumours, versus those from metastatic tumours. There are no significant differences between these cell types: levels of expression in melanocytes and cell lines from primary melanomas of all genes examined fell within the wide range observed in lines derived from metastases (Figure 2; Table 1).

*Correlations in expression levels of p53, c-*myc *and c-*src*-1, and c-*erb*-B2*

The most striking finding of our study is that it has demonstrated significant correlations in melanoma cells between mRNA levels of p53 and c-*myc* ($r = 0.55$, $P < 0.05$), p53 and c-*src*-1 ($r = 0.69$, $P < 0.01$), c-*myc* and c-*src*-1 ($r = 0.68$, $P < 0.01$) and p53 and c-*erb*-B2 ($r = 0.67$, $P < 0.01$). The densitometric values for *src* were very low in some cell lines but it is not these values alone which are responsible for the correlations since the same lines had much higher values for p53 and *myc*. We have considered various ways in which erroneous conclusions could be drawn from densitometric data, but in every case they would *reduce* correlations rather than inflate them. For this reason, and because the *src*, *myc* and p53 results were obtained from three different blots, we believe these correlations to be valid. Such correlations have not been reported before in any cell type so we do not know whether this is a general phenomenon, or restricted to melanoma cells in culture. In either case it implies that a common regulatory factor may be acting to control mRNA levels in this genes in a coordinated manner. Using factor analysis, we calculated that this factor could account for 77%, 62% and 81% of the variance in mRNA levels between lines in c-*src*-1, c-*myc* and p53, and also 30% of the variance in c-*erb*-B2. This 'factor' could be a transcription factor, or could be a physical factor such as ultraviolet irradiation (UVR). UVR causes a transient increase in c-*myc* mRNA in a transformed keratinocyte line (Ronai *et al.*, 1988), and stimulates p53 levels in murine fibroblasts (Maltzman & Czyzyk, 1984). Whether it also affects c-*src*-1 and c-*erb*-B2 levels is not known. Both p53 and c-*myc* are growth-regulated genes and the level of their expression correlates with the proliferation status of the cells (Kelly *et al.*, 1983; Reich & Levine, 1984) but it is unlikely that this alone explains these correlations since (a) all the cell lines were harvested at a similar level of confluency and (b) c-*src*-1 and c-*erb*-B2 have not been shown to be expressed in this way.

In order to evaluate the biological significance of this finding it is important that expression of these genes be

studied in primary melanomas, as well as other melanoma cell lines. In addition, it is necessary to confirm that correlations in mRNA levels are mirrored at the protein level. Understanding the orchestration of gene expression in melanoma cell lines might provide the key to critical events in melanoma initiation and progress.

Materials and methods

Cell culture

Normal foreskin melanocytes were cultured according to the method of Eisinger and Marko (1982). The medium consisted of modified Eagle's medium (Gibco) with 10% heat-inactivated fetal calf serum, penicillin (100 μg ml^{-1}), streptomycin (100 μg ml^{-1}), tetradecanoylphorbolacetate (100 ng ml^{-1}) and cholera toxin (10 mM). The cells were incubated in 5% CO_2 in air at 37°C for up to 15 passages over 6–8 months. Cultures from several different individuals were pooled as soon as they reached confluency in order to isolate RNA.

17 malignant melanoma (MM) cell lines were used in this study: MM96, MM127, MM138, MM170, MM200, MM229, MM409, MM418, MM455, MM472, MM473, MM485, (Goss & Parsons, 1977; Pope *et al.*, 1979), NKI-4 (de Vries *et al.*, 1974), WW, BL, COLO239F (Moore *et al.*, 1980) and Mel13. COLO239F, MM418 and MM200 were derived from primary melanomas. WW was derived from a local recurrence of a melanoma and hence could be either primary or metastatic. Mel13 is of unknown origin. The remainder of the lines originated from metastatic lesions. The cell lines were maintained in RPMI 1640 containing 10% heat-inactivated fetal calf serum, penicillin (100 μg ml^{-1}) and streptomycin (100 μg ml^{-1}) and were incubated in 5% CO_2 in air at 37°C. RNA was isolated as soon as the cells reached confluency.

RNA isolation

Poly-adenylated RNA was isolated according to the method of Gonda *et al.* (1982). Cells were trypsinized and resuspended in STE (100 mM NaCl, 10 mM Tris-HCl, pH 7.5, 1 mM EDTA) with 200 μg ml^{-1} pre-digested proteinase K and 0.5% SDS. They were then homogenized in glass and the homogenate was incubated at 37°C for 1–16 h. Poly-A$^+$ RNA was isolated by adding NaCl to 0.5 M followed by mixing the cell lysate with oligo d(T)-cellulose for 2 h before conventional poly-A$^+$ RNA selection (Aviv & Leder, 1972).

DNA isolation

Genomic DNA was isolated by the 'salting out' method (Miller *et al.*, 1988) from the supernatant remaining after the oligo d(T)-cellulose and bound poly-A$^+$ RNA was spun down from the cell lysates. Further NaCl was added to 1.5 M prior to precipitation of the protein by centrifugation and ethanol precipitation of DNA from the supernatant.

Northern and Southern analysis

7 μg poly-A$^+$ RNA was electrophoresed at 30 V overnight in 1% formamide-formaldehyde gels with 1 × MOPS buffer (Maniatis *et al.*, 1982). RNA samples were prepared with 50% formamide, 2.2 M formaldehyde and 1 × MOPS buffer and heated to 60°C for 10 min prior to loading. RNA was transferred to Zeta-Probe (Biorad) or Hybond N$^+$ (Amersham) membrane in 50 mM NaOH according to the manufacturer's instructions. An RNA ladder (BRL) was used for the estimation of molecular weights. For Southern analysis, 10 μg DNA was digested with restriction enzymes prior to separation in 0.8% agarose gels at 30 V overnight. DNA was transferred, after acid nicking, to Hybond N$^+$ in 0.4 M NaOH (Reed & Mann, 1985).

Plasmids were radioactively labelled by nick translation to approximately 5×10^8 cpm μg^{-1}. Northern filters were hybridized overnight at 55°C in 50% formamide, 2 × SSPE, 1% SDS, 0.5% blotto, 0.5 mg ml^{-1} yeast RNA and 10% dextran sulphate and washed to a stringency of 0.2 × SSC at 65°C. Southern filters were hybridized at 65°C in 2 × SSPE, 1% SDS, 0.5% blotto, 0.5 mg ml^{-1} salmon sperm DNA and 10% dextran sulphate and washed to a stringency of 0.5 × SSC at 65°C. Northern blots were stripped and re-hybridized until the signal strength diminished (up to 12 times).

Several exposures were obtained from each hybridization and the level of signal was quantified by densitometry using a GS300 Transmittance/Reflectance densitometer (Hoeffer Scientific Instruments). Glyceraldehyde-3-phosphate-dehydrogenase (GAPDH) was used as a loading control for the Northern blots. The level of expression was estimated by obtaining the ratio of the intensity of hybridization signal for the probe of interest over that for GAPDH (Thompson *et al.*, 1986).

Table 3 Plasmids used in Northern analyses

Gene	*Plasmid*	*Source*	*Reference*
c-*dbl*	p3-7	human cDNA	Ron *et al.*, 1988
c-*src*	phucsrel	human genomic	Parker *et al.*, 1985
c-*myc*	pMC41RC	human genomic	Dalla Favera *et al.*, 1982
N-*myc*	pNB-1	human genomic	Schwab *et al.*, 1983
L-*myc*	L-myc	human genomic	Nau *et al.*, 1985
c-*erb*-B2	pKX044	human genomic	Semba *et al.*, 1985a
c-*erb*-B2	pHER2-436-1	human cDNA	Coussens *et al.*, 1985
c-*int*-2	SS6	human genomic	Casey *et al.*, 1986
v-*yes*	pv-yes	viral genomic	Semba *et al.*, 1985b
c-*mel*	pEMBL600	human genomic	Padua *et al.*, 1984
c-*hst*	0.78EcoRIhst	human genomic	Koda *et al.*, 1987
p53	pR4-2	human cDNA	Harlow *et al.*, 1985
PDGF-A	PDGF-A	human cDNA	Betsholtz *et al.*, 1986
PDGF-B/c-*sis*	pL335, pL331	human genomic	Dalla Favera *et al.*, 1981
PDGF-R	p131-HK6	murine cDNA	Yarden *et al.*, 1986
EGF-R	pE7	human cDNA	Merlino *et al.*, 1984
TGFα	phTGF1-10-925	human cDNA	Murray *et al.*, 1986
EGF	phEGF121	human cDNA	Bell *et al.*, 1986
gro	pGEM TC 870	human cDNA	Anisowicz *et al.*, 1987
bFGF	pHFL1-7	human cDNA	Kurokawa *et al.*, 1987
t-PA	PA01	human cDNA	Edlund *et al.*, 1983
u-PA	pHUK-8	human cDNA	Verde *et al.*, 1984
GAPDH	pHcGAP	human cDNA	Piechaczyk *et al.*, 1984

Plasmids

The plasmids used are detailed in Table 3.

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