Melanoma Research 1994, 4, pp. 213–223

Cellular pathways leading to melanoma differentiation: therapeutic implications

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The induction of differentiation, as evidenced by benign growth characteristics, dendritic morphology, pigmentation capability, and a mature antigenic phenotype, is an attractive theoretical basis for therapy in human melanoma. Melanoma differentiation can be experimentally induced by modulating intracellular pathways involving protein kinase C, tyrosine kinases, and protein kinase A, or by modulating nuclear transcription with retinoids, DNA-damaging agents, and chemotherapeutic drugs. Other experimental differentiating agents include the amino acid tyrosine, histamine receptor antagonists, polyamine antagonists, dimethylsulphoxide, caffeic acid ester, and butyrate. The mechanisms involved in the actions of many of these agents are beginning to be understood and the pathways are often intersecting; cross-talk in the form of negative and positive feedback loops is extensive. Uncoupling of pathways is also seen, with some agents leading to simultaneous increases in both differentiated and transformed characteristics. While clinical benefits of this approach have so far been sparse, greater understanding of the cellular pathways of differentiation may open new therapeutic options in melanoma.

Key words: Differentiation, melanocyte, melanoma, transcription factor, transformation, protein kinase A, protein kinase C, tyrosine kinase.

Cancer is histopathologically graded according to the cytological state of differentiation: the less differentiated, the more 'atypical' and the higher the grade; the more a tumour cell resembles a normal cell of its own lineage, the less malignant its properties. The apparent 'de-differentiation' of tumour cells may actually reflect their origin from earlier benign precursors with a superimposed malignant phenotype, and reversion to this benign histiotype can be directed in some tumours.[1] Alternatively, therapeutic driving of transformed cells to terminal differentiation, while potentially impairing normal processes such as wound healing, may result in little non-specific killing of normal cells and may thus offer a favourable therapeutic strategy. In human melanocytic tumours, spontaneous complete regression due to terminal differentiation is commonly observed in naevi[2,3] and occasionally in melanomas.[4] Partial spontaneous regression of melanoma is often seen clinically but may actually portend a poor prognosis,[3] suggesting the persistence and selection of non-differentiating, more aggressively proliferating subpopulations. Any therapeutic induction of differentiation will clearly need to be essentially complete and permanent.

Figure 1 depicts current conceptions of melanocytic differentiation. Primary differentiation is an embryologic developmental phenomenon: melanocytes which express the receptor encoded by *c-kit*[5] migrate from the neural crest to the basal epidermis, where the embryonic microenvironment may effect their histiotypic commitment as evidenced by a reversion to a benign phenotype in transplanted murine melanoma.[6] There is currently no evidence of a melanocyte stem cell in the skin, although the melanoblast has been defined as a cell which contains premelanosomes but does not express tyrosinase or melanin.[7] 'Premelanocytes'[8] have been identified which are apparently more differentiated and do express the tyrosinase gene. Undifferentiated melanocytes might be found to have characteristics similar to those of the keratinocyte precursor: undifferentiated morphology, slow cycling but responsive to mitogenic stimuli, and localization in a protected, vascularized and innervated anatomical area.[9]

Differentiated melanocytes are antigenically different from their undifferentiated precursors and they are morphologically dendritic, less able to proliferate in response to stimuli, and contain melanosomes and tyrosinase activity. It should be noted, however, that melanin content, unlike tyrosinase, does not correlate with the stage of melanocyte differentiation,[10] nor does pigmentation itself correlate with melanoma differentiation either clinically[11] or *in vitro*.[12]

Houghton and co-workers,[13] noting difficulties with melanin, morphology, and growth characteristics as markers of melanoma differentiation, have proposed the categorization of melanomas based on antigenic phenotypes into stages which correlate with presumed normal melanocyte development. Many systems of antigenic markers have been proposed to distinguish the level of differentiation of

Supported in part by NCI grant CA 25874.
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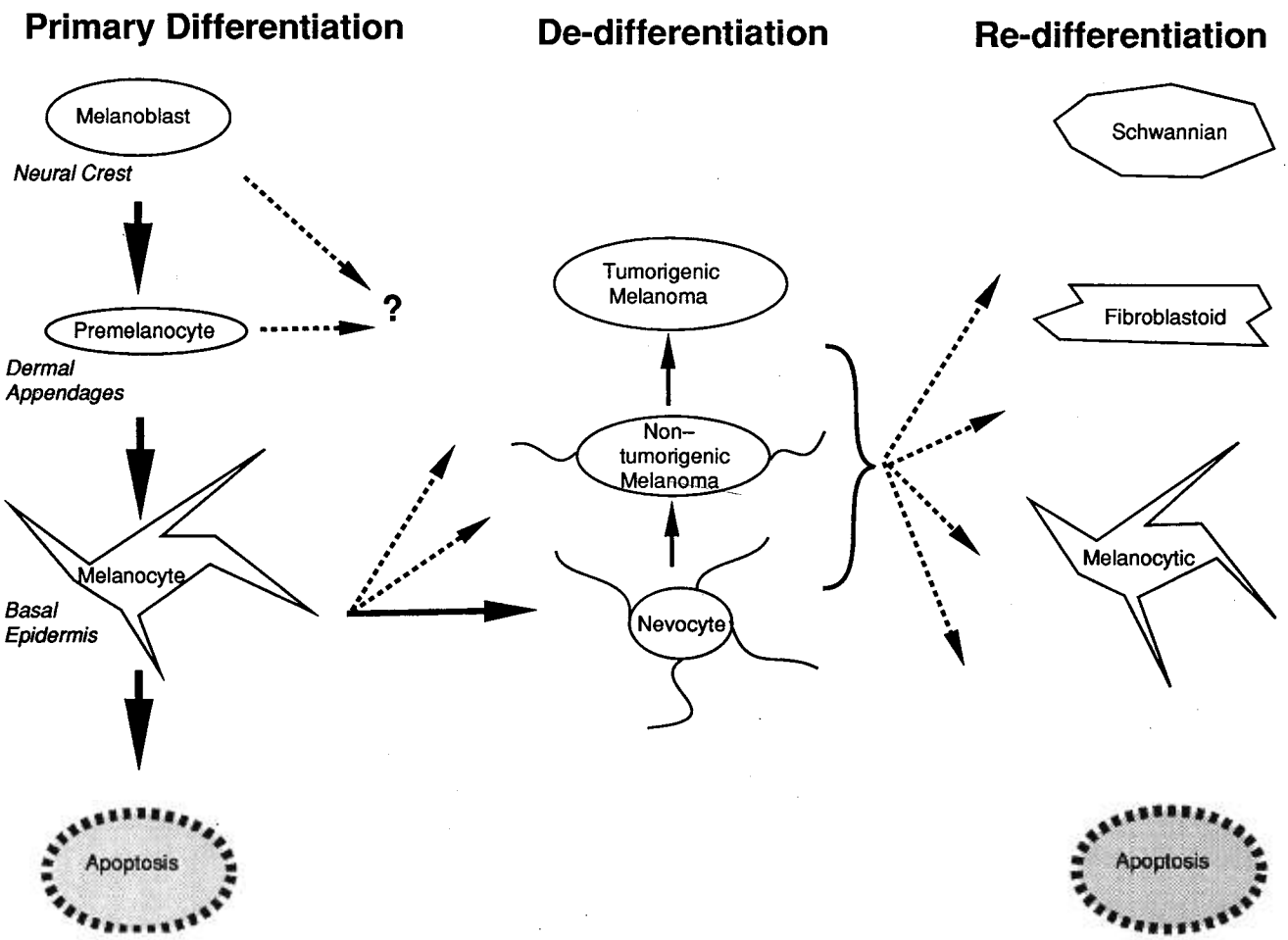

Figure 1. Current concept of the stages of melanocytic differentiation. Left column: 'primary differentiation', maturation or developmental differentiation which correlates with anatomic locations. Middle column: 'de-differentiation', or transformation and tumour progression. Non-tumourigenic melanomas include radial growth phase tumours without invasive or metastatic competence. Right column: 're-differentiation' of transformed cells to either the melanocytic parental phenotype, a new (presumably related) phenotype, or to apoptosis.

both benign and malignant melanocytes.[14-16] Antigens present on both melanomas and melanocytes reflect commitment to the primary differentiation programme, while antigens present on melanomas but absent from melanocytes may reflect tumour origin from an earlier precursor, de-differentiation, or re-commitment to a new, non-melanocytic, phenotype ('re-differentiation', Figure 1). As examples of non-melanocyte phenotypes, desmoplastic melanomas[4] and dermal nevocytes may resemble Schwann cells morphologically and antigenically. Melanomas[17] and naevocytes[18] may also be induced to express a fibroblast-like phenotype.

A developmental concept related to differentiation is that of apoptosis. This genetically determined programme leads to proliferative incompetence, which in mammals involves inactivation of the gene *bcl-2*[19] and perhaps *mcl-1*,[20] and a characteristic cellular self-destructive cascade. However it is still not clear whether this process is the same as terminal differentiation.[21,22]

Melanocytic transformation can be defined by cellular phenotypic traits such as those summarized in Table 1.[23] Since the process of transformation results in apparent de-differentiation, these traits are often considered to be markers of the state of melanocytic differentiation. The transformed melanocytic phenotype can be reversed by fusion of melanoma cells with benign fibroblasts[49] or immortalized melanocytes,[50] suggesting that expression of the genetic programme of differentiation is blocked at multiple sites in melanoma and that the blocks are removed by fusion with the differentiated genome.[51] Cell fusions may also restore cellular transcriptional regulation, the loss of which originally leads to transformation.

The concept of a blocked critical junction in the differentiation programme has been developed in the Drosophila *lethal (2) giant larva* mutation which results in absence of a gene product.[52] This mutation has no effect on the organism unless the product is absent at precisely the right stage of larval development and the right anatomic

Table 1. Biological characteristics of melanocyte transformation

Marker[a]	Comments
Phorbol independence	Auto-production of PKC-dependent mitogens[22,23]
↓ Growth factor dependence	Autocrine bFGF, IL-8 and MGSA[10]
↑ Plasminogen activator	Autocrine bFGF → activation of bFGF receptor → protease secretion[24] → proliferation[25] and invasion[26]
↑ Glucose transport	High-affinity transporter in cell membranes → facilitated glycolosis,[27] ↑ dependence on glutamine[28]
Culture immortality	SV40-T transfection → immortality, partial transformation;[29,30] *ras* transfection → immortality, complete transformation[31]
↓ Anchorage dependence	Altered integrin profile: ↑ α4,[32] ↑ β3, ↓ α6[33] → *src*-related kinase,[34] cytoplasmic alkalinization[35] → anchorage-like growth signal[36]
↓ Contact inhibition	↑ Muc-18,[37] ↑ GD3 ganglioside,[11] ↑ ICAM-1,[38] → deregulated contact-mediated growth signals
↓ Dendritic morphology	↓ Collagen, ↓ laminin,[39] ↓ fibronectin,[40] altered ganglioside expression,[41,42] → ↓ actin[43]
	ABP-280 → submembranous cytoskeletal cross-links[44]
↑ Surface protein mobility	Fibronectin receptor mobility → cell motility[45-48]
	ABP-280 → surface molecule mobility[44]

[a] ↓, decrease; ↑, increase; →, leading to.

location. If the gene is non-functional when needed, its transcriptional machinery is applied elsewhere and cellular transformation occurs.

Pathways of experimental induction of differentiation in melanoma

Many agents have been shown to induce a more differentiated phenotype in melanoma. (Table 2) Few possess the level of efficacy, safety, and specificity needed clinically, but consideration of their mechanisms of action may focus further in-depth investigations.

Signal transduction

Protein kinase C (PKC). Phorbol esters bind to the regulatory domain of most[53] but not all[54] members of the PKC family of serine-threonine kinases.[55] Long-term exposure of murine melanocytes and melanomas to phorbol esters results in PKC autophosphorylation, proteolysis, and migration to the cell membrane.[56] In human melanocytes chronically exposed to optimal mitogenic levels of phorbol ester, PKC activity is enhanced, whereas it is down-regulated after exposure to higher levels.[57] In human melanoma cells, the effect on PKC of chronic exposure to phorbol esters has not yet been established, and the pathways resulting in the observed differentiated melanoma phenotype following phorbol ester treatment[58,59] remain undefined. However there are possible therapeutic targets in this system. For example, a major substrate for PKC is the 80 kD MARCKS (myristoylated alanine-rich C-kinase substrate) family of proteins, which has been implicated in a wide range of activities involving cytoskeletal rearrangements.[60] MARCKS proteins may be involved in establishing the differentiated (dendritic) morphology, anchorage and monolayer dependence, and cell-surface protein mobility observed after phorbol ester treatment.

Protein tyrosine kinases (PTK). PKC is activated by PTK phosphorylation of phospholipase-Cγ.[61] PKC in turn transmodulates (inactivates) the PTK cell-surface epidermal growth factor (EGF) receptor.[62,63] Thus an increase in PKC activity, such as that resulting from stimulation by EGF, decreases mitogenic signals from this PTK. Melanoma cells in culture are initially[64] mitogenically stimulated by EGF or the related transforming growth factor-α (TGF-α), but become less responsive and independent after passage in culture;[65] however, whether these modified responses to EGF are related to PKC activity has not been studied.

Signal modulation by the PTK inhibitor genistein leads to melanoma differentiation.[66] However genistein also decreases intracellular polyamines and nitric oxide production and reverses the action of topoisomerase, which may contribute to the endonuclease-induced internucleosomal damage typical of apoptosis.[67] Thus the mechanism by which genistein affects differentiation remains unclear, and it probably acts at several levels.

Basic fibroblast growth factor (FGF), which plays a central role as a growth factor in the melanocyte system, must be supplied to melanocytes in culture and is pro-

Table 2. Melanoma differentiating agents

Agents	Effects and Comments[a]
Signal transduction	
Phorbol esters[56,57]	MARCKS;[58] ERK → AP-1;[87] block p34^{cdc2} & DNA synthesis[85]
Genistein[64]	↓ Tyrosine kinase, ↓ polyamines, ↓ topoisomerase
Antisense bFGF-R[66]	Phorbol esters partially reverse differentiation
Interferon-β[67]	With ↑ PKC → irreversible differentiation
Dibutyryl cAMP[73,74]	↑ PKA;[70,71] gene modulation[134]
Theophylline[73]	↓ Phosphodiesterase → ↑ cAMP → ↑ PKA
Caffeine[75]	↓ Phosphodiesterase; ↓ cisplatin activity
MSH[76–79]	↑ Adenyl cyclase → ↑ cAMP in murine melanoma
GTP[83]	↑ Adenyl cyclase; ↑ G proteins
Mycophenolic acid, tizofurin[84]	↓ IMP dehydrogenase → ↑ GTP → ↑ G proteins
Nuclear transcription	
Retinoids[95–97]	↑ PKCα;[94] ↓ AP-1 responsive genes[88]
Beta carotene[103]	→ Retinoids; ↓ cAMP; O_2 radical scavenger[104]
[^{3}H]Thymidine[106]	Direct damage to DNA
BrdU[15,107]	Incorporated into DNA → gene modulation
Novobiocin[108]	↓ Topoisomerase[65]
Anthracyclines[110,111]	Damage microtubules; intercalate into DNA;[112] O_2 radicals[113]
AraC, 5-AzadC, aphidocolin hydroxyurea[109]	↓ DNA synthesis; ↑ apoptosis
Other targets	
Tyrosine[114]	↑ Tyrosinase but not melanin
Phenylacetate[28]	↓ Availability of glutamine; modulates genes
Antihistamines[116]	↑ PKC; ↑ PKA[115]
Antipolyamines[123]	↑ Tyrosinase;[120,121] ↑ apoptosis[122]
DMSO[57,124]	Polar molecule which effects multiple mechanisms
Caffeic acid ester[130]	→ Multiple antigen shifts
Butyrate[124,133]	↑ Histone H1°[131,132]

[a] →, leads to; ↑, increases; ↓, decreases.

duced for autocrine stimulation by melanomas.[12] Antisense inhibition of its PTK receptor leads to melanoma differentiation by undefined mechanisms which can be partly antagonized by phorbol ester.[68]

Interferons (IFN) have shown some limited clinical anti-melanoma potential; although interferons up-regulate expression of histocompatability antigens, their variable non-immunological anti-proliferative effects *in vitro* are generally unrelated to differentiation.[69] However IFN-β which may be the most effective in terms of anti-melanoma activity,[70] when combined with PKC activation by the non-phorbol drug mezerein, results in irreversible differentiation of cultured melanoma cells.[71] IFN-β triggers a receptor which sends a transcription signal via a non-receptor PTK.[61] Interferon-induced signalling modifications combined with chemotherapeutic agents may thus offer future efficacious strategies.[72]

The ceramide system, which is associated with tumour necrosis factor-α (TNF-α), induces the intracellular PKC inhibitor sphingosin and also phosphorylates the EGF receptor.[73] This pathway leads to differentiation in promyelocytes,[74] and, possibly, also in melanoma cells.

Cyclic AMP. Many surface hormone receptors transmit their signals via cAMP. There are both positive and negative receptors and either may effect stimulatory or inhibitory guanine nucleotide regulatory (G) proteins at the cell membrane. G-proteins and the similar GTP-binding *ras* proteins control the activity of adenyl cyclase, which converts 5′AMP to cAMP. Adenyl cyclase and phosphodiesterase, which converts cAMP to 5′AMP, control the intracellular level of cAMP. Binding of two cAMP molecules to each of the two regulatory portions of protein kinase A (PKA) accounts for most of the cAMP signal transduction,[75] and substrate phosphorylation.[76]

The cAMP system interacts with PKC at several levels. PKC inhibits positive and some negative surface receptors; it inactivates the inhibitory G-proteins; it activates the catalytic unit of adenyl cyclase; and it inhibits phosphodiesterase. While most of these interactions tend to increase cAMP levels, the net effect is actually cell-specific and dependent on the expression of individual PKC isoforms.[77]

Many agents that increase cAMP levels lead to differentiation of murine melanoma *in vivo* and *in vitro*. Such

agents include the cAMP analogue dibutyryl cAMP,[78,79] the phosphodiesterase inhibitors theophylline[78] and caffeine,[80] and melanocyte stimulating hormone.[81–84] Elevation of cAMP levels in murine melanoma may lead to both a differentiated phenotype and an increased metastatic potential.[81,82,84] The dual effect might be related to varied levels of cross-talk among transcription factors.[85] The extent to which this simultaneous induction of opposing programmes occurs varies with individual cell lines, and its relevance to human melanoma remains unclear.

Guanosine nucleotides. Cyclic guanosine monophosphate (cGMP), the product of nitric oxide-induced guanyl cyclase, is a differentiating agent for promyelocytes,[86] and the cGMP-dependent kinase shares many substrates with PKA. cGMP may either decrease or increase phosphodiesterase activity, and thus cAMP levels, in a specific cell type.[87] The role of cGMP in human melanoma biology has not yet been defined. Guanosine triphosphate (GTP) treatment results in differentiation of murine melanoma,[88] yet a decrease in GTP produced by inhibition of inosine monophosphate dehydrogenase in the purine synthesis pathway, results in differentiation of human melanoma cells.[89] These apparently conflicting results suggest that the species-specific activity of GTP may depend on either stimulatory or inhibitory G proteins and/or the similar *ras* GTP-binding proteins. Definition of any relationship between GTP levels and *ras* activity in melanoma would be interesting in view of the transforming characteristics of *ras* for human melanocytes[33] and the presence of *ras* mutations in approximately 15% of melanomas.[12]

Nuclear transcription

PKC. At the nuclear level, phorbol esters block phosphorylation of $p34^{cdc2}$ in melanoma cells. This is a central regulator of the onset of DNA synthesis,[90] and the block appears to be due to the sustained activation of PKC. The resulting growth arrest is transient and the cells begin to proliferate 20 h after treatment. The ability to restore a regulated cell cycle by this mechanism suggests another potential therapeutic strategy.

Most, but not all, PKC subspecies target IκB, releasing the transcription factor NF-κB.[91] PKC also leads to expression of the generally mitogenic transcription factor AP-1 via phosphorylation of at least one member of the extracellular signal-regulated kinase family (ERKs, also called MAP kinases for mitogen-activated or membrane-associated protein), and phosphorylation of the regulatory portions of c-*jun*.[92] This modulation of transcription factors could account for the activation of proliferation programmes in melanocytes and differentiation programmes in melanoma. These differences may reflect genomic mutation during transformation or cross-talk between structurally or expression-modified transcription factors, which may occur via overlapping, mutually exclusive DNA binding sites or by direct protein–protein interactions.[93,94] The precise transcriptional mechanisms by which PKC switches from driving a programme of proliferation in melanocytes to differentiation in melanomas is another promising area of investigation.

cAMP. PKA phosphorylates cAMP response element binding factors (CREBF), which modulate transcription of genes including c-*fos*, c-*jun*, glucose transporters,[64] and H1° histone.[95]

Retinoids. Transcription is also modulated by retinoids, which are all-*trans*-retinoic acid and its isomers and synthetic analogues.[96,97] Their actions are attenuated by cytoplasmic binding proteins and are mediated by nuclear receptors which are activated by the formation of dimers with themselves or with the receptors for various hormones.[98] These dimers increase the transcription of PKCα, leading to induction of differentiation in murine[99] and in some human[100–102] melanomas. Retinoids also decrease the transcription of AP-1-responsive genes by cross-coupling of the signal transduction pathways.[93] Thus retinoids increase both PKC and its phosphorylated targets while decreasing the mitogenic transcriptional effect of an important target. The therapeutic implications of this double action are evident, and retinoids have indeed been shown to have clinical potential in many malignancies,[103,104] including melanoma.[105,106] An increase in PKC transcription and simultaneous cross-talk between AP-1 and retinoic acid receptors results in a reversal of some of the transformed phenotypic properties of melanoma cells, except that a differentiated, dendritic morphology is not established.[100,102] This may argue against phosphorylation of MARCKS as a sufficient precursor event to cytoskeletal changes. Other actions of retinoids, for which the significance in melanoma has yet to be determined, include modulation of transcription of the homeobox family of transcriptional regulators and increased transcription of such possibly differentiation-related genes as H1° histone,[95] laminin β1, c-*jun*, tissue plasminogen activator, and various growth factors.[107]

β-Carotene, a natural source of vitamin A, is oxidized to retinoids, which may explain its differentiating effect in cultured melanomas.[108] Carotenoids are also potent scavengers of oxygen radicals, and this may allow them to protect the genome from DNA-damaging mutagens.[109] Both carotene and retinol decrease cellular activity

of adenyl cyclase[108] suggesting a potential role for these compounds in the differentiation pathway related to cAMP.

DNA. Many agents that modify DNA have been implicated in melanoma differentiation. Triggering of DNA repair mechanisms or direct arrest of the cell cycle may result in apoptosis,[21] or may create a selective disadvantage, allowing the emergence of differentiating clones which are unaffected by the agent due to low initial levels of DNA synthesis. The actual mechanisms by which these agents induce differentiation are unknown and none have so far achieved therapeutically satisfactory anti-melanoma activity.

Direct injury to DNA can be induced by UV irradiation and by melanin itself,[110] but whether these agents result in differentiation is unknown. Direct damage by radioactive thymidine, however, has produced irreversible differentiation of a human melanoma cell line.[111]

The thymidine analogue 5'bromodeoxyuridine (BrdU) is incorporated into DNA, where it can induce a gene encoding a specific *trans*-acting DNA-binding factor which then acts in tissue-specific fashion to suppress or enhance other genes.[112] In murine melanoma, BrdU reversibly reduces tumourigenicity and induces a flattened (not dendritic) morphology, increased anchorage dependence, decreased proliferation, and decreased transcription of tyrosinase.[112] Similar results were found in human melanoma along with markedly decreased transcription of c-*myc*.[17] These effects demonstrate induction of an incomplete or modified differentiation programme in melanoma and the ability to separate dendritic morphology and tyrosinase from other elements of melanocytic differentiation.

DNA synthesis can be inhibited by drugs such as novobiocin,[67,113] and cytosine arabinoside (AraC), aphidocolin, hydroxyurea, and 5-aza-2'-deoxycytidine (5-AzadC),[114] all of which induce a differentiated melanoma phenotype as well as apoptosis.

Anthracycline antibiotics such as doxorubicin in murine cells[115] and daunomycin in humans,[116] may induce melanoma differentiation by undetermined mechanisms but have little clinical anti-melanoma activity. They are intercalated into nucleic acids and inhibit DNA repair,[117] damage microtubules,[116] and generate free oxygen radicals.[118]

Miscellaneous compounds inducing melanoma differentiation

Amino acids and derivatives. High-dose tyrosine induces dendritic morphology, anchorage independence, and increased tyrosinase levels, but not pigmentation, in a human melanoma line.[119]

Tumour cells are more dependent than normal cells on the amino acid glutamine. Phenylacetate conjugates with plasma glutamine and is active against many tumours, including melanoma. Phenylacetate also effects differentiation associated with down-regulation of c-*myc* in leukemic cells,[30] though this has not yet been reported in melanoma.

Histamine, the product of decarboxylation of the amino acid histidine, is the ligand for three specific surface receptors which, by association with different G proteins, activate PKA, PKC, calcium, and ion channels. Histamine also activates serotonin and other related receptors; and the histamine which is retained intracellularly may initiate cellular proliferation.[120] These relationships are so promiscuous that the specific mechanisms responsible for differentiation in cell culture[121] and the clinical efficacy[122] of histamine receptor antagonists in melanoma are difficult to establish.

Polyamines, products of decarboxylation of the amino acid ornithine, have been implicated as either inhibitors or stimulants of growth or differentiation in various cell types.[123] Ornithine decarboxylase is increased by phorbol esters in murine carcinogenesis models,[124] and inhibitors of ornithine decarboxylase, such as α-difluoromethyl ornithine (DFMO), lead to decreased proliferation and increased tyrosinase and melanin in murine melanomas.[125,126] Murine melanomas also undergo apoptosis in response to high levels of oxidized polyamines when transplanted to an embryonic limb bud.[127] Similarly, polyamine antagonists have been shown to have antitumour effects in human melanoma.[128] Polyamines may therefore have therapeutic potential, although whether or not they are associated with differentiation pathways in the human system remains to be determined.

Dimethylsulphoxide (DMSO). The polar molecule DMSO induces phenotypic changes in human melanomas, but, like retinoids, does not induce a dendritic morphology.[59,129] The mechanisms involved are unclear; in other systems, DMSO has been shown to scavenge oxygen radicals, modulate a transcriptional promoter[130] and c-*myc* translation,[131] effect a post-translational decrease of the SV40 T antigen[132] and c-*myb* oncoproteins,[133] and increase polyamine synthesis.[134]

Caffeic acid phenethyl ester (CAPE). The naturally occurring product CAPE induces dendritic morphology and growth inhibition in a human melanoma cell line while increasing expression of antigens characterizing both

more- and less-differentiated phenotypes. This agent may thus have a dual therapeutic role as a differentiator and, by inducing surface antigen shifts, an immunomodulator.[135]

Butyrate. Sodium butyrate is a differentiating agent for many cell types. In murine melanoma it induces reversible differentiation associated with transcription of histone H1°, which stabilized chromatin and is usually associated with irreversible, terminal differentiation.[136,137] Expression of histone H1° is promoted by retinoids and by cAMP,[95] but it is unknown whether butyrate exerts its effects via these or other pathways. In human melanoma butyrate results in a spindle (rather than dendritic) morphology; it reduces expression of a melanosomal antigen but does not increase pigmentation,[123] and it induces an antigen which is otherwise found only on benign nevus cells.[138]

Conclusions

The interactions among the various pathways implicated in melanoma differentiation are summarized in Figure 2. Signalling is modulated by PKC, PTKs and cAMP/PKA. PKC, which phosphorylates the MARCKS substrate,[60] also inactivates the PTK EGF receptor[62,63] and is itself activated by PTK phosphorylation of phospholipase-Cγ.[61] The TNF-α/sphingosin pathway inhibits PKC and also phosphorylates the EGF receptor.[73] In the cAMP/PKA pathway, PKC inhibits both positive and negative surface receptors, inhibitory G-proteins, and phosphodiesterase while activating adenyl cyclase.[77] Both inhibitory and stimulatory G proteins depend on GTP, and cGMP may either increase or decrease phosphodiesterase activity.[87]

Nuclear regulatory factors of differentiation are produced

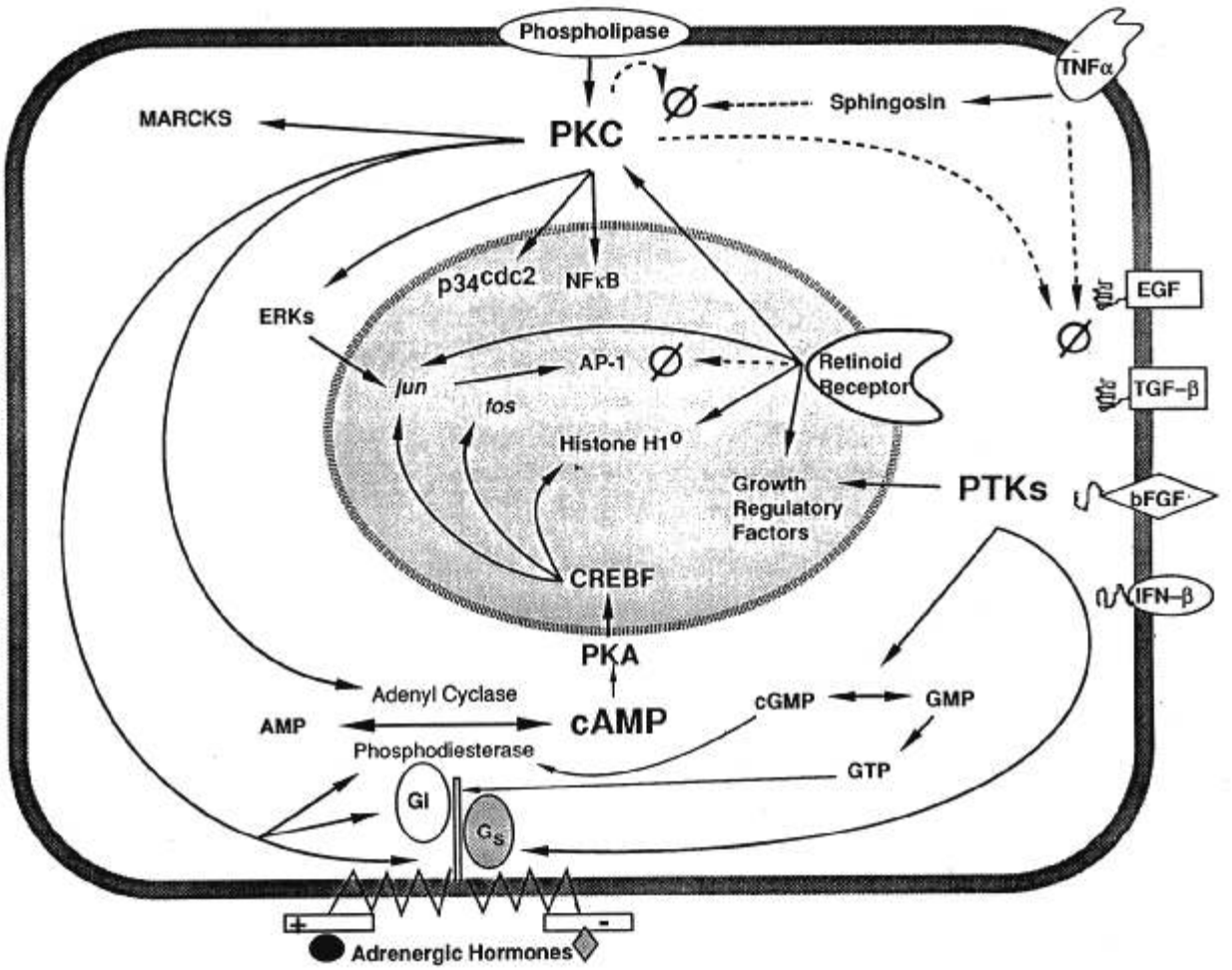

Figure 2. Inter-related pathways of melanoma differentiation. Activation of surface membrane receptors results in signal transduction modulated by interactions of PKC, PTKs and cAMP/PKA. All of these plus the nuclear retinoid receptor result in cross-talking nuclear transcriptional regulatory factors. See text for complete descriptions. →, positive/activating pathway; → ∅, negative/down-regulating pathway.

by PKC, PTKs, cAMP/PKA, and retinoid receptors. PKC, which blocks the $p34^{cdc2}$ DNA synthesis factor,[90] activates NFκB[91] and activates AP-1 via the ERKs pathway.[92] AP-1 is also increased by the cAMP/PKA-dependent CREBF[139] and its activity is decreased by retinoids.[93] Retinoids also increase transcription of PKC,[99–102] c-*jun*, growth-regulatory factors,[107] and the H1° histone.[95] Histone H1° stabilizes chromatin and is associated with terminal differentiation;[136,137] it is also increased by cAMP/PKA.[95] The complexity of these interrelationships argues against the possibility of any simple linear process that results in universal, irreversible differentiation.

The interactions between these pathways are fluid and there are many examples of uncoupling between an overall differentiating effect and associated characteristics such as decreased proliferation or increased pigmentation. Simultaneous increases in both tumour differentiation and metastatic progression, as seen with cAMP enhancers in murine melanoma,[85] may represent a tool for further investigation as well as a potential hazard for any therapeutic applications.

The expanding knowledge of these mechanisms may well lead to therapeutic approaches which can either drive tumour cells to terminal differentiation or definitively turn back the clock of transformation to a benign melanocytic phenotype.

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(Received 25 November 1993; accepted 21 April 1994)