

# Microarray analysis sheds light on the dedifferentiating role of agouti signal protein in murine melanocytes via the Mc1r

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The melanocortin-1 receptor (MC1R) is a key regulator of pigmentation in mammals and is tightly linked to an increased risk of skin cancers, including melanoma, in humans. Physiologically activated by  $\alpha$ -melanocyte stimulating hormone ( $\alpha$ MSH), MC1R function can be antagonized by a secreted factor, agouti signal protein (ASP), which is responsible for the lighter phenotypes in mammals (including humans), and is also associated with increased risk of skin cancer. It is therefore of great interest to characterize the molecular effects elicited by those MC1R ligands. In this study, we determined the gene expression profiles of murine melan-a melanocytes treated with ASP or  $\alpha$ MSH over a 4-day time course using genome-wide oligonucleotide microarrays. As expected, there were significant reductions in expression of numerous melanogenic proteins elicited by ASP, which correlates with its inhibition of pigmentation. ASP also unexpectedly modulated the expression of genes involved in various other cellular pathways, including glutathione synthesis and redox metabolism. Many genes up-regulated by ASP are involved in morphogenesis (especially in nervous system development), cell adhesion, and extracellular matrix-receptor interactions. Concomitantly, ASP enhanced the migratory potential and the invasiveness of melanocytic cells *in vitro*. These results demonstrate the role of ASP in the dedifferentiation of melanocytes, identify pigment-related genes targeted by ASP and by  $\alpha$ MSH, and provide insights into the pleiotropic molecular effects of MC1R signaling that may function during development and may affect skin cancer risk.

pigmentation | skin cancer

A major determinant of the pigment phenotype of the skin is the melanocortin-1 receptor (MC1R), a G protein coupled receptor (GPCR) that regulates many functional aspects of melanocytes, including their dendricity, melanogenesis, and proliferation (1, 2). MC1R function can be activated by  $\alpha$ -melanocyte-stimulating hormone ( $\alpha$ MSH), a POMC-derived peptide whose production in the skin (3) is increased by exposure to UV radiation (UV) via a p53-dependent mechanism (4). This interaction triggers increased melanin production (5, 6) and stimulates the repair of DNA photoproducts caused by UV (7–9), a phenomenon also induced by forskolin (10). It is therefore not surprising that MC1R polymorphisms are associated with a lighter pigment phenotype, including red hair/fair skin (11), with poor tanning responses (12) and an increased risk for melanoma and other skin cancers (13, 14).

MC1R signaling can be inhibited by the product of the agouti locus, agouti signal protein (ASP in mice, ASIP in humans), which acts as an inverse agonist of the murine Mc1r (15, 16). In mice, ASP is expressed in skin and testis and during embryogenesis. The spatiotemporal expression of ASP in skin is responsible for the agouti hair phenotype (a pheomelanin band against a dark eumelanin background) and for the pale coloration on the neck, breast, and ventral surface (17). A high degree of polymorphism in the promoter of the agouti gene (18) and epigenetic modifications (19) result in a wide diversity of mutant phenotypes, ranging from all black (a/a) to a uniform yellow coat (A<sup>y</sup>/a). Ubiquitous overexpression of ASP in lethal yellow mice

(A<sup>y</sup>/a) is responsible for a pleiotropic syndrome characterized by obesity, enhanced linear growth, metabolic derangements, earlier mortality, and tumorigenesis (20). The mechanism(s) by which ASP induces tumorigenesis has not been elucidated, but several models have been proposed (21–24).

ASIP is widely expressed in human tissues (skin, heart, reproductive tract, fat tissue, liver, and kidney) (25), and although its functions in humans are poorly characterized, as in mice, the agouti locus regulates human pigmentation (26–29). In light of the correlation of MC1R variants with increased skin cancer risk, and considering the tumorigenic effects of ASP, one can expect that ASP would contribute to melanoma and/or other skin cancer risk. Interestingly, 2 different groups reported an association between variants on chromosome 20q11.22, which lies in the vicinity of ASIP, and cutaneous melanoma and basal cell carcinoma (30, 31). Although further studies will be necessary to confirm whether ASIP is the causal variant, a growing body of evidence supports this hypothesis.

Therefore, it is of major interest to further characterize the role played by ASP in regulating melanocyte function and the mechanism(s) of MC1R signaling through its ligand interactions. A recent expression profiling study was performed in the skin of mice carrying a loss of function mutation for Mc1r (Mc1r<sup>e/c</sup>) after UV irradiation and identified a set of Mc1r-dependent genes involved in the cell cycle and oncogenesis (32). Earlier, Voisey *et al.* reported the effects of ASIP on human melanoma cells transfected to over-express ASIP (33). Only 5 new targets of ASIP were identified which may be due, at least in part, to the A2058 melanoma cells used in their study, which express low levels of MC1R (34), or the efficacy of ASP secretion, which was not reported. In this study, we used oligonucleotide microarray technology to identify previously undescribed downstream targets of ASP and  $\alpha$ MSH in melanocytes over a 4-day time course.

ASP down-regulated an impressive panel of genes encoding proteins required for the pigmentary functions of melanocytes, uncovering the dedifferentiation process that occurs, whereas  $\alpha$ MSH stimulated the expression of those genes. Surprisingly, the gene expression profile of melanocytes treated with ASP also revealed significant increases in the expression of genes involved in developmental and cell adhesion processes. Thus, the database generated in this study is useful not only to identify targets involved in melanogenesis but also to provide insights of the role of ASP in

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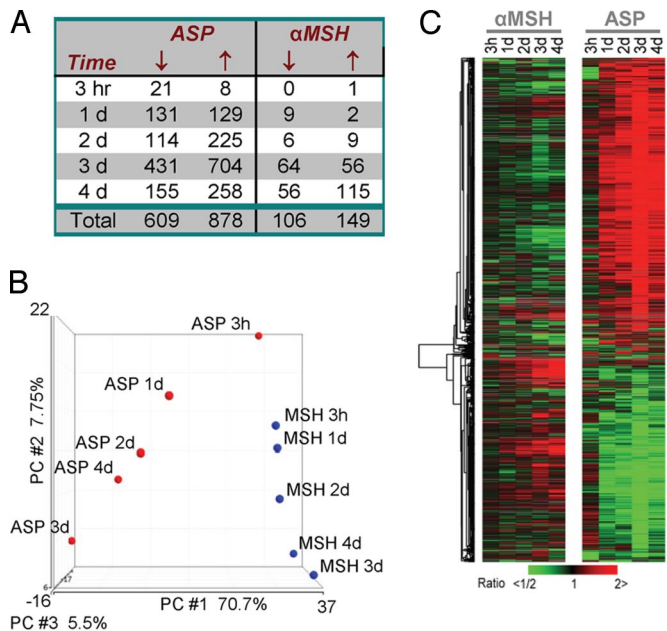
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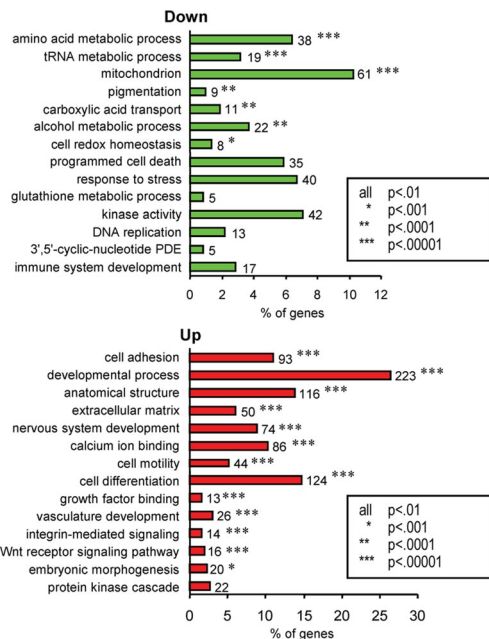
**Fig. 1.** Overview of transcriptome changes after treatment of melanocytes with ASP or  $\alpha$ MSH. The microarray experimental design was based on competitive hybridizations of ASP- or  $\alpha$ MSH-treated vs. untreated melanocytes after 3 h, 1 d, 2 d, 3 d, and 4 d of treatment. (A) Number of genes differentially altered in response to ASP or  $\alpha$ MSH by at least 1.5-fold for at least 1 time point of the 5 assessed. (B) PCA analysis of 1,715 differentially expressed genes after treatment with ASP or  $\alpha$ MSH ( $P < 0.01$  and 1.5-fold at any 1 time point) for which data were present for all time points. Projection of the 3 principal components covering the highest variance (84% of the total variance) shows drug-dependent variation along PC#1 (70.7%), whereas time-dependent changes are observed along PC#2 (7.7%) and PC#3 (5.5%). (C) Hierarchical clustering of 1,638 genes altered either by ASP or by  $\alpha$ MSH using an average linkage algorithm, underscores the overall inverse regulation of the genes by  $\alpha$ MSH relative to ASP. Distance metric is 1 correlation. Horizontal stripes represent genes, and columns show experimental samples. Clustering was performed only on genes, whereas samples were ordered by drug and time. Logarithmic values of treated to untreated expression ratios are shown in the heat map using red and green color codes for up- and down-regulation, respectively. Black indicates no change, and gray represents missing data.

other cellular functions and thus a better understanding of MC1R signaling.

## Results and Discussion

**Overview of Transcriptome Changes Induced by ASP or  $\alpha$ MSH.** To elucidate molecular mechanisms controlled by Mc1r, we undertook gene expression profiling of melanocytes cultured in the presence of ASP or  $\alpha$ MSH for 3 h, 1 d, 2 d, 3 d, or 4 d. Gene expression levels in ASP- or  $\alpha$ MSH-treated cells relative to untreated controls were determined using microarrays that contained a total of 38,784 oligos targeting murine genes. Transcripts were selected as significantly altered when the average ratio calculated from the 6 replicates per time point was  $\geq 1.5$  (false discovery rate,  $< 1\%$ ) for at least 1 time point out of the 5 (the entire DNA microarray database is deposited at [www.ncbi.nlm.nih.gov/geo](http://www.ncbi.nlm.nih.gov/geo), GSE 14089). A total of 1,487 and 255 unique probes were significantly modulated by ASP or by  $\alpha$ MSH, respectively (Fig. 1A). Even though fewer genes were significantly altered by  $\alpha$ MSH compared with ASP (Fig. S1), both ligands had overall opposite effects, as highlighted by principal component analysis (PCA) mapping (Fig. 1B), by heat map clustering of the complete set of altered genes (Fig. 1C), or by comparing genes significantly and inversely regulated by both ligands (Fig. S2).

The database for annotation, visualization, and integrated discovery (DAVID), which is based on Gene Ontology molecular function (MF), biological process (BP), cellular component (CC),



**Fig. 2.** Summary of the functional categories of genes significantly enriched in response to ASP. Analyses were performed individually on 609 and 878 significantly ( $P < 0.01$ ) down- or up-regulated genes by ASP, respectively, using DAVID. All GO groups demonstrated enhanced statistical representation (EASE score value  $< 0.01$ ). Bars represent the proportion of genes involved by each category, for which statistical significance and number of genes is indicated. For complete breakdown of significantly enriched gene ontology groups, see Table S1.

and KEGG pathways, was then used to identify enriched function-related gene groups characterizing the different datasets generated. Data mining analysis of genes significantly altered by  $\alpha$ MSH (271 unique IDs) or commonly altered by both ASP and  $\alpha$ MSH (110 unique IDs) led to similar functional categories compared with the ASP signature (see Table S1 for a list of genes).

The majority of genes differentially expressed in ASP-treated cells were regulated in a similar manner within most of the identified categories, except for apoptosis, cell proliferation, calcium ion binding, MAPK signaling, kinase activity, and cyclic-nucleotide phosphodiesterase activity, where variable numbers of genes were up- or down-regulated. Therefore, we report in Fig. 2 the enriched functional groups of genes altered by ASP, within each of the 2 sets of up- or down-regulated genes. Genes down-regulated by ASP included those involved in biosynthesis, e.g., pigmentation, translation, transporters, alcohol metabolism, DNA replication, GSH anabolism, and mitochondrial redox abilities. However, genes up-regulated by ASP had a significant enrichment in differentiation and developmental biological processes, in cell adhesion and motility, and in extracellular matrix–receptor interactions.

**ASP Down-Regulates Pigment-Related Genes and Their Encoded Proteins.** Given the primary role of Mc1r in controlling pigmentation and melanocyte function (35–37), we initially validated the microarray data by characterizing the effects of ASP on known pigment-specific genes. Among the 279 mapped loci known to influence color in mice (38), 113 have been cloned to date, and 24 of those are significantly altered by ASP in melanocytes (Table 1). *Mitf*, *Tyr*, *Tyrp1*, *Dct*, and *Si* were down-regulated, whereas *Tcf4* was up-regulated. Previous studies reporting a similar effect of ASP on those genes (15, 16, 39, 40) were consistent with our data. We identified 16 other pigment-related genes whose transcription was repressed by ASP and 2 transcription factors which were up-regulated (*Lef1* and *Tcfap2a*/Ap2a).

**Table 1. Genes encoded by coat-color loci that are significantly altered by ASP**

Gene	Mutant Phenotype	Function	ASP 3 h		ASP 1 d		ASP 2 d		ASP 3 d		ASP 4 d	
			<i>P</i> value		<i>P</i> value		<i>P</i> value		<i>P</i> value		<i>P</i> value	
<i>Mitf</i> , <i>microphthalmia</i> transcription factor	Small eyes, white hair	Melanocyte development	-1.13	< 0.01	-0.06	0.74	-0.35	0.10	0.04	0.67	-0.10	0.41
<i>Sfnx1</i> , <i>sideroflexin</i>	Flexed tail	Melanocyte development	-0.01	0.93	-0.14	0.36	-0.47	0.10	-0.96	< 0.01	-0.68	0.06
<i>Gpnmb</i> , <i>Glycoprotein</i>	Iris pigment dispersion	Melanosome component	-0.18	0.32	-0.76	< 0.01	-1.16	< 0.01	-1.37	< 0.01	-1.16	< 0.01
<i>Rab38</i> , <i>RAS oncogene family</i>	Chocolate hair	Melanosome component	0.16	0.32	-0.43	0.01	-0.47	0.04	-0.79	< 0.01	-0.76	< 0.01
<i>Matp</i> , <i>membrane-associated transporter</i>	White hair	Melanosome component	-0.33	0.15	-1.15	< 0.01	-0.79	< 0.01	-1.33	< 0.01	-1.06	< 0.01
<i>Pmel17</i> , <i>silver protein</i>	Silver hair	Melanosome component	-0.23	0.18	-0.90	< 0.01	-0.89	< 0.01	-1.40	< 0.01	-1.13	< 0.01
<i>Slc24a5</i> , <i>solute carrier family 24 # 5</i>	Golden hair	Melanosome component	-0.01	0.94	-1.01	< 0.01	-1.10	< 0.01	-1.45	< 0.01	-1.32	< 0.01
<i>Tyr</i> , <i>tyrosinase</i>	White hair	Melanosome component	-0.32	0.05	-0.56	< 0.01	-0.68	< 0.01	-0.82	< 0.01	-1.18	< 0.01
<i>Typr1</i> , <i>tyrosinase-related protein</i>	Brown hair	Melanosome component	-0.12	0.53	-0.66	< 0.01	-0.65	< 0.01	-0.91	< 0.01	-0.68	0.03
<i>Typr2/Dct</i> , <i>tyrosinase-related protein 2</i>	Gray hair	Melanosome component	-0.16	0.31	-1.21	< 0.01	-0.99	< 0.01	-1.39	< 0.01	-1.06	< 0.01
<i>Ggt1</i> , $\gamma$ - <i>glutamyltranspeptidase 1</i>		Melanin synthesis/switch	-0.14	0.14	-0.19	0.22	-0.43	0.03	-0.60	< 0.01	-0.27	0.12
<i>Slc7a11</i> , <i>solute carrier family 7 # 11</i>	Subtle gray hair	Melanin synthesis/switch	-0.19	0.61	-0.90	< 0.01	-1.18	< 0.01	-2.04	< 0.01	-1.18	0.12
<i>Hps3</i> , <i>Hermansky-Pudlak syndrome gene 3</i>	Cocoa hair	Melanosome trafficking	0.02	0.84	-0.28	< 0.01	-0.44	0.01	-0.72	< 0.01	-0.56	0.04
<i>Melan-A (Mlana, Mart1)</i>		Melanosome trafficking	0.16	0.50	-0.27	0.27	-0.72	< 0.01	-0.81	< 0.01	-0.61	< 0.01
<i>Oca1</i> , <i>ocular albinism type 1</i>	Unpigmented eyes	Melanosome trafficking	0.04	0.29	-0.63	< 0.01	-0.71	< 0.01	-0.72	< 0.01	-0.77	< 0.01
<i>Oca2</i> , <i>ocular albinism type 2 (P)</i>	Pink eyes, grey hair	Melanosome trafficking	0.09	0.51	-0.51	0.02	-0.75	0.04	-0.79	< 0.01	-0.73	< 0.01
<i>Rab27a</i> , <i>RAS-associated protein</i>	Ashen hair	Melanosome transport	0.12	0.35	-0.40	0.03	-0.54	0.02	-0.92	< 0.01	-0.65	0.04
<i>Cited1</i> , <i>Cbplp300-interacting transactivator 1</i>		Unknown	0.07	0.41	-0.65	< 0.01	-0.59	0.07	-0.78	nd	-0.78	< 0.01
<i>Brcal</i> , <i>breast cancer 1</i>		Unknown	0.01	0.98	-0.49	0.04	-0.37	0.04	-0.67	< 0.01	-0.81	< 0.01
<i>Tcfap2a</i> , <i>transcription factor AP-2<math>\alpha</math></i>		Neural crest development	0.03	0.88	0.00	0.98	0.47	0.03	0.70	< 0.01	1.01	0.10
<i>Itf2/Tcf4</i> , <i>transcription factor 4</i>		Unknown	0.13	0.12	1.20	< 0.01	0.83	< 0.01	1.23	< 0.01	0.97	< 0.01

Coat-color genes significantly modulated by ASP for at least 1 time point of the 5 assessed. Values indicate averaged logarithmic ratios to the base 2 of 6 replicates for each time point, followed by *P* values.

The levels of most proteins encoded by those genes were evaluated in a time- and/or dose-dependent manner where specific antibodies were available, and they confirmed the microarray results (Fig. 3). *Mitf*, the master melanogenic transcription factor, was significantly decreased by ASP within 3 h and remained much lower than the control at all other time points analyzed, whereas it was up-regulated by  $\alpha$ MSH. Among the *Mitf* targets characterized, the melanogenic enzymes (*Tyr*, *Typr1*, and *Dct*) and other proteins participating in melanosome function (*Si*, *Mart-1*, *Gpr143*, and *Rab27a*) were significantly down-regulated by ASP, as were *Met* and *Cdk2*. The inhibition by ASP of other pigment genes, including *Rab38*, *P* and *Gpnmb*, raises interesting speculation concerning their possible regulation by *Mitf*.

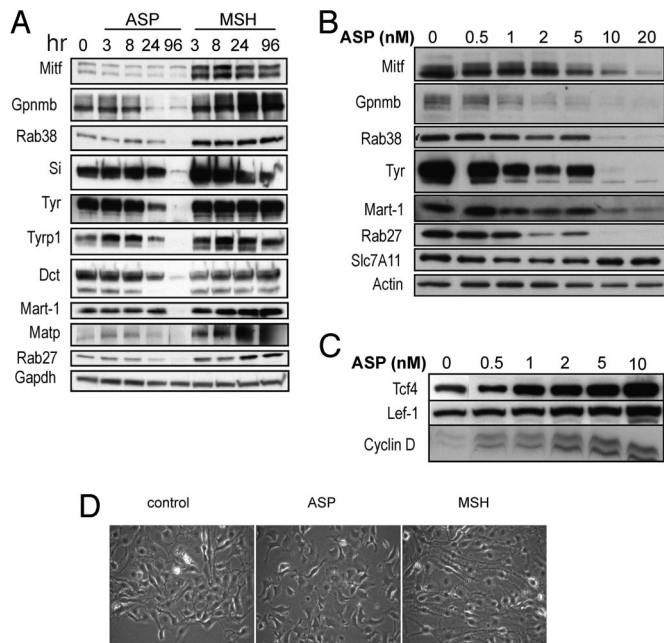
A growing number of solute carrier (*Slc*) genes have been identified that play important roles in regulating mammalian pigmentation and in determining skin color (41–44). Several recent studies are in agreement with the inhibitory role of ASP on many of the *Slc* genes that were differentially expressed. Newton *et al.* reported the up-regulation of MATP by NDP-MSH, which is abolished in melanocytes expressing RHC variants for MC1R (45). *Slc24a5*, also regulates melanogenesis (42, 46) and is down-regulated in yellow quails overexpressing *agouti* (47). In addition to the inhibitory effects of ASP on proteins related to melanosome maturation and function, ASP also modulated genes encoding proteins involved in melanosome transport, such as *Rab27a*, *Rab38*, and *Hps3*.

It is tempting to speculate that most melanosomal proteins implicated in melanin production are also likely down-regulated by ASP. Thus, we report in Tables S2 and S3 genes differentially expressed in response to ASP that encode melanosomal proteins identified by proteomics (48). For instance, *Slc24a4* was consistently decreased by ASP and up-regulated by  $\alpha$ MSH and thus appears to be a strong candidate to regulate melanogenesis. Indeed, 2 genome-

wide association studies (44, 49) have recently linked this gene with human hair and skin pigmentation.

Therefore, ASP alters the basic function of melanocytes by inhibiting the expression of factors involved in various steps of the pigmentary cascade, i.e., melanosome biogenesis and maturation, pigment synthesis, and intracellular transport. The effects of ASP observed on coat color genes are Mc1r-dependent, because the products of those genes were not down-regulated by ASP in melanocytes carrying a loss-of-function mutation at the Mc1r locus (Mc1r<sup>ec</sup>) (data not shown). Concomitantly, ASP severely altered melanin synthesis within 1 d in melan-a melanocytes (35) and elicited noticeable morphological changes (Fig. 3C). Overall, these data confirm the inhibitory effect of ASP on melanocyte differentiation, a phenomenon observed in murine melanoblasts (39, 50). Thus, the activation of MC1R by  $\alpha$ MSH stimulates the differentiation of melanocytes to augment their biological functions whereas ASP turns off this process, reversing Mc1r signaling and triggering melanocyte dedifferentiation.

**Potential Effects of ASP on Intracellular Signaling Pathways, Redox Function, and DNA Repair.** Interestingly, among the genes regulated by ASP were a number of intermediates in the MAPK pathway, the majority of them being up-regulated, whereas their inhibitors [such as the dual specificity protein phosphatases (DUSP)] were down-regulated. The up-regulation of MAPK signaling is known to influence the coordinated loss of expression of several melanocytic antigens in melanoma cells (51). Our analysis also revealed significant increases in expression of genes belonging to the Wnt signaling pathway (Table S3) in response to ASP. Among those genes, we used immunoblotting to confirm the up-regulation of *Tcf4*, *Lef-1*, and *cyclin D* (Fig. 3). This induction of Wnt-related genes by ASP is quite surprising, because Wnt has not only been shown to promote melanocytic differentiation, but also to serve as a major



**Fig. 3.** Effects of ASP and  $\alpha$ MSH on pigment-related protein expression levels. The ASP-induced down-regulation of gene products known to play roles in melanogenesis were assessed by Western blot analysis to confirm time- (A) and dose-dependent (after 3 d of treatment) (B) effects of ASP on Mitf, Dct, Tyr, Tyrp1, Gpnmb, Rab38, Slc7A11, Matp, Mart-1, Rab27a, and Si. The time-dependent effects of  $\alpha$ MSH were analyzed and showed the up-regulation of the same genes compared with ASP. (C) Modulation of components of the Wnt pathway in response to ASP. Immunoblotting of Tcf4, cyclin-D, and Lef-1 showing the dose-dependent increase at the protein level of selected targets found up-regulated in the microarray dataset. (D) Effects of ASP on cell morphology.  $\alpha$ MSH induced a more dendritic phenotype compared with control, whereas ASP modulated the shape of melanocytes toward a melanoblast-like shape [20 $\times$  magnification after 4 d treatment with ASP (10 nM) or with  $\alpha$ MSH (100 nM)].

factor in development during embryogenesis (52–54). Tcf4 has been shown to be overexpressed in agouti mice during the transient expression of ASP and to be induced by ASP in melanocytes, where it acts as a negative transcriptional regulator of Mitf, Tyr, Tyrp1, and Dct (55). Therefore, Tcf4 up-regulation seems to favor Mitf inhibition triggered by ASP, although the mechanisms of induction remain unclear.

The decrease in expression of Mitf and its downstream targets elicited by ASP may also arise at least in part from the down-regulation of the SRY HMG box Sox9 (56) and from the inactivation of PKA. The microarray dataset reveals the up-regulation by ASP of genes encoding (i) Prkar1a and Prkar1b, 2 regulatory subunits of PKA inactivating its catalytic domains; (ii) Pkia and Pkib, 2 competitive inhibitors of PKA which interact with the catalytic subunit of the enzyme (both down-regulated by  $\alpha$ MSH); and (iii) Cri, the inhibitor of Crebbp (or its homologue P300), which is required in the transcriptional activation complex induced by PKA. The regulatory domain of PKA (RIIB) mediates the secondary effects of ASP in the lethal yellow syndrome (obesity and linear growth) (57), and the catalytic domain of PKA (Prkaca) has been associated with the shift from pheomelanin to eumelanin synthesis (58). Furthermore, the induction of Mitf and Tyr by cAMP in neural crest cells prompts their differentiation into melanocytes (59), a phenomenon mainly dependent on PKA, which further supports the concept that the inhibition of PKA signaling plays a key role in the inhibition of differentiation induced by ASP.

The altered transcription of genes related to oxidative stress and mitochondria, including ATF4, suggests that ASP affects the redox abilities of melanocytes. For instance, ASP down-

regulated genes involved in synthesis (Gclc, Nags), reduction (Gsr) or transfer (Gsta1, Gsta2, Gstp1) of glutathione, which is relevant to the imbalance of thiols that correlates with ASP expression in mice (60). In light of the ability of  $\alpha$ MSH to reduce oxidative stress and DNA damage after UV exposure (61), those previously unidentified targets become of great interest in understanding this phenomenon.

Also regulated by ASP were 12 distinct transcripts that encode proteins involved in the repair of DNA damage. BRCA1 and Ddit3 (also known as Gadd153) were down-regulated by ASP and might play important roles in the nonpigment related effects of MC1R function on DNA repair (7–9). We recently showed that treatment of reconstructed skin with forskolin, which increases cAMP and activates PKA, enhanced the expression of XPC and the repair of UV-induced DNA damage (10). Thus, identification of genes/proteins down-regulated by ASP, whose functions are related to DNA repair, are of obvious interest. In support of this concept is the down-regulation of *NR4A3*. Members of the NR4A family play a role in the repair of UV-induced DNA damage after stimulation of human melanocytes by a superpotent  $\alpha$ MSH analogue (9). Further, the induction of *NR4A* by that MSH derivative was abrogated in RHC variants of MC1R (9).

**Genes Up-Regulated by ASP: Insights into MC1R Functions?** Genes that are normally expressed during morphogenesis/organogenesis or are involved in cell adhesion/communication were significantly enriched among the transcripts up-regulated by ASP (Fig. 2 and Table S4). Examples of genes contributing to various processes during embryonic morphogenesis, including *Tcfap2a*, *CTGF*, *ErbB3*, *Efnb2*, and *N-cadherin*, were confirmed to be up-regulated by ASP at the protein and mRNA levels (Fig. S3). *Tcfap2a* is a retinoic acid-inducible gene required during neural crest development and formation of its derivatives (62) and has been implicated in cancer. The ASP-mediated induction of *Tcfap2a* observed in melanocytes also occurred in B16F1 murine melanoma cells, and interestingly, *Tcfap2a* was dramatically increased in human melanocytes carrying the mutation R160W or D294H (2 MC1R variants associated with melanoma) compared with wild-type MC1R (data not shown). *ErbB3*, a member of the epidermal growth factor receptor (EGFR) family implicated with causation or sustenance of various cancers, including those originating from melanocytes and from brain cells (63), was not only increased by ASP but was also decreased by  $\alpha$ MSH.

Surprisingly, 74 of the developmental genes increased by ASP are involved in the development of the nervous system, including axon guidance molecules and genes involved in neurogenesis, such as *ErbB3*, *Ablim1*, *Foxd1*, *Uchl1*, *Amigo1*, and *EphB2*, and genes expressed during migration of neural crest cells (NCC), such as *sema3f*, *sema3c*, *Nrp2*, *gbx2*, and *Efnb1*. Other developmental categories include genes involved in skeletal, bone and cartilage development, such as *Sox4*, *Sox6*, and *Postn*, and kidney, muscle development, and vasculogenesis (*Ctgf*, *Pdgfa*, *Pdgfb*, *Mef2c*, and *Efnb2*).

ASP-treated melanocytes seem to have undergone a switch from their differentiated melanocytic state toward a pattern of genes displaying neuronal specification and other developmental features characteristic of their precursors, the NCC (64). In a similar manner, melanocytes isolated from quail embryos and cultured in the presence of endothelin 3, which is crucial for the development of 2 NC-derived lineages (melanocytes and enteric nerve cells), are able to revert into glia through a neural crest-derived glial-melanocytic progenitor, as elegantly shown by Dupin *et al.* (65). The plasticity of NCC has been well established and interestingly, the reminiscence of a multipotent phenotype is observed in the most aggressive melanoma (66). Indeed, it is thought that highly metastatic melanoma cells reexpress embryonic genes and by doing so acquire the ability to behave like their precursors did, i.e., proliferate or migrate and invade. Further, Gupta *et al.* showed that the



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