Published in final edited form as:
Pigment Cell Melanoma Res. 2008 August ; 21(4): 415–428. doi:10.1111/j.1755-148X.2008.00478.x.

G-protein-coupled receptors and melanoma

Hwa Jin Lee, **Brian Wall**, and **Suzie Chen***
Susan Lehman Cullman Laboratory for Cancer Research, Department of Chemical Biology, Ernest Mario School of Pharmacy, Rutgers, The State University of New Jersey, Piscataway, NJ, USA

Summary

G-protein-coupled receptors (GPCR) are the largest family of receptors with over 500 members. Evaluation of GPCR gene expression in primary human tumors identified over-expression of GPCR in several tumor types. Analysis of cancer samples in different disease stages also suggests that some GPCR may be involved in early tumor progression and others may play a critical role in tumor invasion and metastasis. Currently, >50% of drug targets to various human diseases are based on GPCR. In this review, the relationships between several GPCR and melanoma development and/or progression will be discussed. Finally, the possibility of using one or more of these GPCR as therapeutic targets in melanoma will be summarized.

Keywords

G-protein-coupled-receptors; oncogenes; melanocytes; melanoma

Introduction

The processes of cell growth and cell differentiation are determined by a combination of cellular microenviron-mental influences, as well as inter- and intracellular interactions. Included in the many consequences of altered regulation of either cellular growth or differentiation is tumorigenesis. Melanoplasia is a complex disease whose etiology may involve several possible pathways controlling normal melanocytic growth. Cancer research conducted in the past several years has provided convincing evidence that mutations in proto-oncogenes and/or tumor suppressor genes, which control growth and proliferation of normal cells, can give rise to neoplastic transformation. Proto-oncogenes may be activated by a number of mechanisms including mutational changes in the coding region or over-expression caused by genomic amplification or transcriptional up-regulation. For tumor suppressor genes, deletions and/or mutations in both copies are required to abrogate its activity, in most cases.

The occurrence of melanoma in the US is rising rapidly and, on its present course, the lifetime risk will reach one in 75 among Caucasians in the next 5 years (American Cancer Society, Cancer Facts and Figures, 2007). Despite a great deal of clinical and molecular efforts directed towards the treatment and prevention of this disease, the precise genetic lesions leading to melanoma remains to be discovered. The availability of an animal model system that recapitulates the onset and progression of a given human disease is a valuable tool. Normally spontaneous, malignant melanomas are a rare occurrence in rodents. However, melanomas have been induced by carcinogenesis protocols in several mouse model systems (Bradl et al., 1991; Chin et al., 1997; Iwamoto et al., 1991; Kato et al., 1998; Klein-Szanto et al., 1994;

*Address correspondence to S. Chen, suziec@rci.rutgers.edu.

Krimpenfort et al., 2001; Mintz et al., 1993; Noonan et al., 2003; Otsuka et al., 1998; Powell et al., 1999; Takayama et al., 1997). In these model systems, the anomalous expression of a multi-functional cytokine or an oncogene is needed for tumor formation and, in some instances, introduction of an exogenous carcinogen is also required. Furthermore, tumorigenesis in these model systems occurs in a wide variety of tissues in addition to cutaneous melanoma. Nevertheless, these mouse models have been very valuable for the assessment of melanoma susceptibility genes and molecular events leading to the disease (Bardeesy et al., 2000).

G-protein-coupled receptors as oncogenes

G-protein-coupled receptors (GPCR) have a major role in regulating physiological functions, including neurotransmission, hormone and enzyme release from endocrine and exocrine glands, immune responses, cardiac- and smooth-muscle contraction and blood pressure regulation. Their dysfunction contributes to some of the most prevalent human diseases, as reflected by the fact that GPCR represent the target, directly or indirectly, of 50–60% of all current therapeutic agents (Lundstrom, 2006; Pierce et al., 2002; Schlyer and Horuk, 2006).

All GPCR share a characteristic core composed of seven-transmembrane α-helices that weave in and out of the membrane (Pierce et al., 2002) allowing them to be regulated by many agonists/antagonists. Activated GPCR typically control cellular physiology by releasing the signaling potential of inactive heterotrimeric G-proteins. These inactive heterotrimers consist of a guanine diphosphate-bound Gα subunit, which maintains a high affinity for a G$\beta\gamma$ functional monomer. Upon activation by a cognate ligand or signal, a GPCR catalyzes the exchange of GTP for GDP on the Gα subunit resulting in a decreased affinity of Gα for G$\beta\gamma$. This alteration causes dissociation of the subunits (Hamm, 1998) or rearrangement of the heterotrimers (Bunemann et al., 2003) allowing Gα and G$\beta\gamma$ to interact with and to modulate the activities of a diverse and growing repertoire of effector molecules (Cabrera-Vera et al., 2003). Ultimately, the G-protein-coupling specificity of each receptor determines the nature of its downstream signaling targets (Neves et al., 2002). GPCR have traditionally been linked to many of the functions performed by differentiated, post-mitotic cells. However, GPCR are also expressed in proliferating cells, and contribute to embryogenesis, tissue remodeling and repair, inflammation, angiogenesis, normal cell growth and cancer. Indeed, many potent mitogens such as thrombin, lysophosphatidic acid (LPA), gastrin-releasing peptide (GRP), endothelin and prostaglandins stimulate cell proliferation by acting on their cognate GPCR in various cell types (Dorsam and Gutkind, 2007; Marinissen and Gutkind, 2001; Mills and Moolenaar, 2003; Rozengurt et al., 2002). Furthermore in 1986 the discovery of *MAS*, the first GPCR with oncogenic potential, provided a direct link between cellular transformation and GPCR (Young et al., 1986). Surprisingly, in contrast to most oncogenes identified at that time, *MAS* did not harbor activating mutations. Further work showed that wild-type GPCR can become tumorigenic when exposed to an excess of locally produced or circulating ligands/agonists (Gutkind et al., 1991; Julius et al., 1989) while in other GPCR, mutations in key conserved residues can render GPCR to have transforming activity even in the absence of their ligands (Allen et al., 1991; Pollock et al., 2003a). Currently, aberrant functioning of GPCR pathways have been shown to lead to several important human diseases and a large body of evidence links aberrant G-protein signaling to cancer development and progression. For example, a recent examination of publicly available gene expression data identified a variety of GPCR that are over-expressed in diverse types of cancer tissues (Li et al., 2005). In addition, GPCR have a central role in tumor-induced angiogenesis and tumor metastasis. Many cancers metastasize to specific organs with an incidence much greater than would be expected from the circulatory pattern between the primary tumor site and the secondary organs. Lymph node metastasis is one of the first elements of tumor cell dissemination in most human cancers. Tumor cell migration and metastasis share very similar patterns in movement with leukocytes, which is critically regulated by GPCR and their ligands, particularly G-protein-linked

chemokine receptors and chemokines. Enhanced levels of chemokines have been detected in secondary organs prior to evidence of metastasis. Organ-specific metastasis is likely mediated by aberrant expression of G-protein-linked chemokine receptors in cancer cells concomitant with the release of chemokines from the secondary organs resulting in promoting tumor cell proliferation, migration and neovascularization in tumor tissue (Balkwill, 2004; Ben-Baruch, 2008; Chambers et al., 2002). Taken together these results suggest that tumor cells use several strategies to satisfy their increasing needs for nutrients and oxygen, including the modification of their local environment and switching their gene-expression programs to produce angiogenic factors. Many solid tumors rely on GPCR, such as thrombin, prostaglandin and S1P receptors, as well as CXCR2 and CXCR4 chemokine receptors, to elicit an angiogenic response either by acting on endothelial cells for stromal components directly or through the regulation of the release or activity of other angiogenic mediators such as vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (bFGF), by stromal and immune cells (Richard et al., 2001). Taken together, these results suggest that interfering with these receptors and their downstream targets might provide an opportunity for the development of new, mechanism-based strategies for cancer diagnosis, prevention and treatment.

GPCR and melanoma

Melanocytes arise from the neural crest, the differentiation of which is crucial to vertebrate development. Neural crest cells develop into melanoblasts, the precursors of melanocytes, undergo massive proliferation, and migrate beneath the ectoderm along a dorso-lateral pathway. Similar to melanocyte development, tumorigenesis involves morphogenesis, movement and proliferation. Several recent reports hypothesize that the aggressive metastatic characteristic of melanoma may be attributed to the neural crest origin of melanocytes (Gupta et al., 2005; Kim et al., 2006). Increasing evidence suggests that GPCR are involved in tumorigenesis and metastatic progression of melanoma. The role of some GPCR in melanoma tumor biology including transformation, proliferation, migration, invasion, and angiogenesis are summarized below.

Melanocortin type 1 receptor (MC1R) and melanoma

The melanocortin type 1 receptor (MC1R) is a key determinant of the broad phenotypic diversity in mammals. MC1R preferentially binds to the pro-opiomelanocortin derived peptides α-melanocytes stimulating hormone (α-MSH) and adrenocorticotropic hormone (ACTH) resulting in the activation and crosstalk of multiple downstream signaling cascades, predominantly in a cAMP-PKA-dependent manner (Newton et al., 2007a,b; Rees, 2003; Roberts et al., 2006, 2007, 2008; Smith et al., 2008). In addition to being an important contributor in the proliferation and differentiation of melanocytes, MC1R has also been implicated in the development and growth of melanoma cells (Cheng et al., 2007; Kadekaro et al., 2003). Abdel-Malek et al. (2000) have demonstrated toxic responses to UV in human melanocytes that carry loss of function mutations in MC1R. On the contrary, melanocytes with functional MC1R can tolerate UV-induced cell death better, which may initial several early events in cell transformation leading to tumor development (Abdel-Malek et al., 1999, 2000; Kadekaro et al., 2005a; Suzuki et al., 1999). In addition, a large number of natural polymorphisms of MC1R have been identified in different populations. Association between hair and skin color phenotypes, melanoma and non-melanoma skin cancers with particular MC1R polymorphisms have been studied by many investigators. Results from these studies have shown that MC1R variants, particularly those variants most strongly associated with red hair color and fair skin (RHC), increase risk of melanoma (Gerstenblith et al., 2007; Kennedy et al., 2001; Matichard et al., 2004; Palmer et al., 2000; Valverde et al., 1996). Epidemiology studies showed correlations between MC1R RHC variants and two commonly mutated genes in melanoma, CDKN2A and BRAF in melanoma patients (Box et al., 2001; Goldstein et al.,

2005, 2006, 2007; Landi et al., 2006). Box et al. (2001) reported the increased risk of melanoma of three common MC1R variants with CDKN2A mutation(s). These three MC1R variants have been associated previously with red hair, fair skin and sensitive skin to UV light. Recently, Smith et al. (2008) reported possible mechanisms of enhanced melanoma risk by MC1R variants. They showed induction of three NR4A receptors as one of the immediately downstream transcriptional targets of MC1R in melanocytes. NR4A receptors belong to the nuclear receptor superfamily of transcriptional regulators and a large sub-group of orphan receptors. In addition, they also demonstrated that melanocytes with the homozygous RHC MC1R variants exhibited a decrease in NR4A induction in response to MC1R-agonist. Prior to UV-irradiation, primary human melanocytes were transfected with siRNA to two of the NR4A receptors. As a consequence, the ability to remove UV-induced cyclobutane pyrimidine dimmers was reduced in these melanocytes. These results suggest that activation of NR4A receptors by stimulated MC1R play critical roles in protecting melanocytes to UV-induced DNA damage.

Endothelin receptors (EDNR) and melanoma

The endothelin (ET) system is a family of three similar small peptides (ET-1, 2, 3; Inoue et al., 1989; Itoh et al., 1988), two G-protein coupled receptors (EDNRA, EDNRB; Ihara et al., 1992a,b; Ishikawa et al., 1994) and two proteinases (ECE-1, ECE2; Kedzierski and Yanagisawa, 2001). Endothelins and EDNRB have been demonstrated to participate in melanocyte transformation and melanoma progression (Bittner et al., 2000; Lahav, 2005; Lahav et al., 2004; Soufir et al., 2005, 2007; Thirumaran et al., 2006). Expression profiling of human melanoma biopsies and cell lines indicates that EDNRB is over-expressed and therefore associated with an aggressive phenotype and is used as a tumor progression marker (Bittner et al., 2000). Other studies that have supported this notion included UV-induced activation of the ET-1/EDNRB pathway down-regulated by E-cadherin, a suppressor of melanoma cell invasion, resulting in an enhancement of melanoma invasive capability (Hsu et al., 2000; Jamal and Schneider, 2002). When levels of ET-1 were elevated by transfection of exogenous ET-1 into human melanoma cells, an increase in cell growth was detected (Berger et al., 2006). Possible mechanisms that may be contributed by excess ET-1 in the increase of survival of melanocyte and inhibition of UV-induced apoptosis is by activating the phosphatidylinosito 3-kinase (PI3K)-Akt pathway (Kadekaro et al., 2005b).

Blockade of EDNRB by several known antagonists including Bosentan, a dual antagonist for endothelin receptors, BQ788, a specific antagonist of EDNRB, or another antagonist A-192621, increased melanocyte differentiation markers, apoptotic cells and decreased cell growth were detected in in vitro cultured cells and in vivo xenografts in nude mice. In addition, suppression in the accumulation of several key factors in tumor metabolism and tumor progression including HIF-1α, VEGF, and MMP-2 were also detected (Berger et al., 2006; Kefford et al., 2007; Lahav, 2005; Lahav et al., 1999, 2004). A phase II clinical trial with Bosentan as monotherapy in patients with stage IV metastatic melanoma resulted in stabilization of the disease in six of 32 patients (Kefford et al., 2007).

Wnt/frizzled receptor and melanoma

Signaling through the Wnt pathway begins with Wnt ligands, which are a family of secreted, cysteine-rich peptides that act by binding to their cell surface transmembrane receptors in the frizzled family to initiate the downstream signaling cascades. Three distinct pathways have been identified to participate in Wnt signaling and possible crosstalk between these pathways has been suggested (Polakis, 2000; Weeraratna, 2005). Earlier studies showed that, in developing mouse embryos, the absence of both Wnt1 and Wnt3a resulted in a deficiency in neural crest derived cells, including melanocytes, suggesting that both Wnt1 and Wnt3a are

critical in the growth and the expansion of melanocytes (Ikeya et al., 1997). However, Dunn et al. (2000, 2005) used cell lineage-directed gene targeting approach and showed that, contrary to previous reports, Wnt1 and Wnt3a have distinct roles in promoting the development of neural crest-derived melanocytes. Several studies have demonstrated that the consequences of dysregulation of one or more of the three Wnt signaling pathways are malignant transformation of cells leading to onset of various human cancers, including melanoma (Reya and Clevers, 2005; Weeraratna, 2005). Gene array expression profiling has identified the correlation between Wnt5a and a specific cluster of highly aggressive melanoma samples (Bittner et al., 2000). In addition, transfection of Wnt5a cDNA into less aggressive noninvasive melanoma cells results in increased invasiveness in these recipient cells (Bachmann et al., 2005; Dissanayake et al., 2007; Weeraratna et al., 2002). Constitutive activation of the Wnt signaling pathway can be induced by deregulation of one or more of components in the Wnt signaling pathway including β-catenin, Brn2 and naturally endogenous Wnt inhibitory factor 1 (WIF-1; Rimm et al., 1999; Omholt et al., 2001; Goodall et al., 2004; Lin et al., 2007). In other tumors, the role of Wnt5a is controversial, it is detected to be up-regulated in lung, breast and prostate cancers but down-regulated in pancreatic and urothelial carcinomas, where Wnt5a is considered to act as a tumor suppressor (Crnogorac-Jurcevic et al., 2001; Iozzo et al., 1995; Olson et al., 1997). This is in line with the findings that Wnt5a signals through not only the canonical pathway via stabilization of β-catenin but also different non-canonical pathways (Itoh et al., 2005; Topol et al., 2003). In a panel of benign and malignant melanocytic tumors, expression of both Wnt5a and its receptor frizzled were significantly reduced from benign nevi to melanomas; this is consistent with a tumor suppressor role (Bachmann et al., 2005). How Wnt signaling is involved in melanoma development and progression still remains largely unknown thus requiring further evaluation.

Chemokine receptor (CXCR) and melanoma

The chemokines are divided into four structural types: CXC, CC, C and CX_3C. All chemokines signal via receptors belonging to the seven transmembrane domain family of G protein-coupled receptors (Kakinuma and Hwang, 2006; Murphy et al., 2000; Rot and von Andrian, 2004). Chemokine receptors have been detected in a variety of human cancer cell lines including melanoma cells. In addition to being expressed in melanoma cells, CXCR3 and CXCR4 were shown to be functional in these cells and may contribute to cell proliferation (Robledo et al., 2001). Recent reports suggest that the expression of chemokines and chemokine receptors in melanoma cells may contribute to the ability of these cells to escape tumor surveillance, which may explain preferential patterns of melanoma metastasis to sites such as lymph nodes and lung. In human and murine melanoma cell lines, expression of chemokine receptor has been reported to facilitate site-directed metastases. For example, expression of functional CXCR4 in either B16 mouse melanoma cells or human melanoma cells shows enhanced metastases in lung, liver, lymph nodes and dermal layer of skin (Cardones et al., 2003; Murakami et al., 2002; Scala et al., 2006). Other chemokine receptors including CCR7 and CCR10 have also been linked to lymph node or skin metastases (Forster et al., 2001; Kawada et al., 2004; Murakami et al., 2003, 2004; Simonetti et al., 2006; Takeuchi et al., 2004). Recently, Seidl et al. (2007) reported that expression profiles of chemokine receptors are substantially different in melanocytic lesions and cultured melanoma cell lines. Expression of CXCR6 was detected in primary melanomas and melanoma metastases, but absent in melanoma cell lines and congenital nevi. Only two chemokines receptors, CXCR4 and CCR1, were consistently detected in melanocytic lesions. Moreover, a significant increase in levels of CCR1 was found in melanoma. These investigators suggest that expression profiles of metastatic and adjacent normal tissue likely to share some chemokine receptors at similar expression levels, but only a select few receptors are then up-regulated during tumor progression to metastases (Seidl et al., 2007).

Protease-activated receptor-1, thrombin receptor (PAR-1) and melanoma

Protease-activated receptor-1, thrombin receptor (PAR-1) is a unique G-coupled protein receptor that belongs to the protease-activated receptor family. Activation of PAR is mediated enzymatically by serine protease through the cleavage of the amino terminal domain. Activated PAR have been implicated in numerous biological process, including inflammation (Asokananthan et al., 2002), coagulation (Ruf et al., 2003), mitogenesis (Madamanchi et al., 2001), and cell proliferation (Darmoul et al., 2003). In addition to its roles in normal vascular regulation and tissue remodeling, PAR-1 also facilitates tumor invasion, angiogenesis, and metastasis by modulating the expression of cell adhesion molecules, matrix-degrading proteases and the secretion of angiogenic factors in several cancers including melanoma (Even-Ram et al., 1998, 2001; Fischer et al., 1995; Nierodzik et al., 1992, 1998; Zain et al., 2000). In stable PAR-1 transfected mouse melanoma cells, activation of PAR-1 was shown to promote synthesis and secretion of functional vascular endothelial growth factor proteins, which are highly effective in eliciting tumor angiogenesis (Yin et al., 2003). Furthermore, B16 mouse melanoma cells, when treated with a specific thrombin inhibitor, argatroban, demonstrated suppressed cell migration and metastasis in vivo (Asanuma et al., 2004). Recent studies demonstrated that PAR-1 expression is altered in melanomas in comparison with dysplastic nevi and primary melanoma. The metastatic potential of some human melanoma cell lines was found to correlate with the expression of PAR-1 (Massi et al., 2005; Tellez and Bar-Eli, 2003; Tellez et al., 2003, 2007). A recent study reported that immunohistochemistry for PAR-1 in a panel of 240 malignant melanoma cases showed that PAR-1 can be used as a supplement to Breslow thickness and ulceration as a prognosis indicators for melanoma. In addition, expression of PAR-1 was the best marker of recurrence risk in the study (Depasquale and Thompson, 2008).

Platelet-activating factor (PAF)–PAF receptor and melanoma

Platelet-activating factor (PAF) is a potent pro-inflammatory phospholipid, which is normally produced when neutrophils, macrophages, lymphocytes, and endothelial cells are stimulated by mediators of inflammation (Prescott et al., 2000; Robert and Hunt, 2001; Zimmerman et al., 2002). In addition to the authentic PAF, several PAF-like oxidized phospholipids are produced as byproducts of oxidative fragmentation of cellular phospholipids. These by-products have nearly identical arrays of biological activities as the authentic PAF and when activated, members of this large family mediate their action through the PAF receptor (PAFR). PAFR is a G-protein-coupled-membrane-spanning molecule that engages in multiple signaling pathways which promote tumor growth, angiogenesis and metastatic progression (Balkwill and Mantovani, 2001; Coussens and Werb, 2002; Deo et al., 2004; Mantovani, 2005; Pikarsky et al., 2004; Prescott et al., 2000; Stafforini et al., 2003; Zimmerman et al., 2002).

Im et al. (1996) were the first group to demonstrate the role PAF in melanoma metastasis. They showed that PAF enhanced lung colonization of inoculated B16F10 mouse melanoma cells (Im et al., 1996). Further studies on the pro-proliferative activity of PAF/PAFR were performed with PAFR-over-expressing transgenic mice. In these transgenics, hyper-proliferative alterations in the epidermis were evident as early as 2 weeks after birth. The hyperplasia in keratinocytes was accompanied by hyper-pigmentation and an increase in the number of dermal melanocytes in the ear and tail, and consequent development of melanocytic tumors late in life (Ishii et al., 1997; Sato et al., 1999).

More recently, expression of PAFR was detected in eight-cultured human melanoma cell lines regardless of their metastatic potential (Melnikova and Bar-Eli, 2007; Melnikova et al., 2006). In addition, this group also showed that PAF/PAFR mediated its pro-invasion activity through induction of expression of matrix metalloproteinase (MMP) 2 and MMP-2 activator,

membrane type 1 MMP by stimulation of two key transcription factors, cAMP-response element binding protein (CREB) and activating transcription factor (ATF-1) in human melanoma cells. Treatment with antagonist of PAFR (PAC4248) abolished melanoma metastases (Melnikova and Bar-Eli, 2007; Melnikova et al., 2006). However, growth of solid tumors derived from colon 26 or Ehrlich cells in PAFR null mice was not altered. In these tumor cell xenografts, in addition to lack of inhibition in tumor cell growth, angiogenesis of these xenografts was not altered; only a decrease in some aspects of inflammation were detected (Ferreira et al., 2007; Ishii and Shimizu, 2000). In view of the discrepancies in results with respect to tumor growth and angiogenesis in the absence of a functional PAFR, perhaps differential mechanisms may mediate genetic manipulation (gene knockout) versus pharmacological reagents (PAFR antagonists). This suggests that PAF/PAFR may exert its effects differently in different types of tumors and tumor microenvironment. Furthermore, in PAFR knockout mice, the possibility remains that the inoculated tumor cells still have the ability to synthesize PAFR and being responsive to PAF either produced by the injected cells or from the PAFR knockout host.

Metabotropic glutamate receptor 1 (GRM1) and melanoma

Metabotropic glutamate receptors (mGluRs) are members of the G protein-coupled receptor (GPCR) super-family (Hermans and Challiss, 2001; Pin and Duvoisin, 1995). mGluRs have been shown to play important roles in the mammalian central nervous system, such as learning and memory, neuronal development, and neurodegeneration (Dhami and Ferguson, 2006; Riedel et al., 2003). The natural ligand for mGluRs is glutamate, the predominant excitatory neurotransmitter of the central nervous system and is involved in several central neuronal functions. Glutamate exerts its modulatory effects on neuronal excitability and synaptic transmission by interacting with a family of glutamate receptors. In addition to metabotropic glutamate receptors, there are also the ionotropic glutamate receptors, which include the ligand-gated cation channels NMDA (*N*-methyl-D-aspartic acid), AMPA (α-amino-3-hydroxy-5-methyl-sioxazole-4-propionate) and kainate receptors. Eight distinct mammalian mGluRs have been identified and divided into three subgroups based on sequence homology, intracellular signal transduction pathways and pharmacological properties (Dhami and Ferguson, 2006; Pin and Duvoisin, 1995). Group I mGluRs include GRM1 and GRM5 (To conform to the format of nomenclatures for mouse and human, ‘Grm1’ and ‘GRM1’ refers to mouse and human respectively. In some cases, both may be interchangeable.), they are positively coupled to G_a/q11 proteins. Activation of GRM1/GRM5 stimulates polyphosphoinositide hydrolysis via the formation of inositol 1, 4, 5- triphosphate (IP3) and diacylglycerol (DAG). These second messengers, in turn, stimulate intracellular calcium release and activate protein kinase C (PKC; Aramori and Nakanishi, 1992; De Blasi et al., 2001; Francesconi and Duvoisin, 1998). Thus, these mGluRs can trigger signaling cascades and modulate the activity of ion and ligand-gated channels through functional coupling with transduction pathways such as phospholipase C, adenylyl cyclase, phospholipase A2, phopsholipase D (PLD), tyrosine kinases, extracellular signal-regulated protein kinases and mitogene-activated protein kinase kinase (Aramori and Nakanishi, 1992; Bertrand et al., 2002; Bruno et al., 1999; Busca and Ballotti, 2000; Busca et al., 2000; Choe and Wang, 2002; Ferraguti et al., 1999; Francesconi and Duvoisin, 1998; Iacovelli et al., 2003; Kingston et al., 1998, [1999]; Littman et al., 1995; Marin and Chen, 2004; Ohashi et al., 2002; Skinner et al., 2001; Thandi et al., 2002; Valenti et al., 2002). Results obtained from biochemical studies on Group I mGluRs in heterologous cellular systems showed them to be very similar to the metabotropic receptors observed in brain slices (cortex, hippocampus, etc.) and primary neuronal cultures (Conn and Pin, 1997; Pin and Duvoisin, 1995), confirming the functionality of mGluRs in heterologous cells. Although GRM1 and GRM5 are highly homologous, they have distinct levels of expression and tasks in the central nervous system (Battaglia et al., 2001; Bordi and Ugolini, 1999; Pisani et al., 1997; Valenti et al., 2002). In addition to the central nervous system, differences in tissue distribution and

functions have been reported for GRM1 and GRM5 (Battaglia et al., 2001; Frati et al., 2000; Storto et al., 2000). GRM5, but not GRM1, is found in melanocytes and hepatocytes where it is involved in cell death and inappropriate stimulation and in development of anoxic damage, respectively (Frati et al., 2000; Storto et al., 2000). Atkinson et al. (2006) reported that activation of second messengers in GRM1 is very sensitive to agonist concentration, in contrast, GRM5 mediated activation of second messengers was insensitive to the concentration of agonist. In addition, our laboratory has demonstrated previously that melanoma development in our transgenic models is independent of Grm5 (Marin et al., 2005). We showed that aberrant expression of Grm1 in melanocytes was sufficient to drive melanomagenesis in Grm5 null mice (Marin et al., 2005).

Depending on the cell type of a heterologous system, GRM1 has been shown to have the ability to couple to multiple second messenger systems IP3, DAG and cAMP in the presence of GRM1 agonists (Aramori and Nakanishi, 1992; Francesconi and Duvoisin, 1998). Signaling cascades mediated by GRM1 have been extensively studied in the central nervous system, as well as in heterologous cells transfected with exogenous cDNA of GRM1. In heterologous systems, GRM1 can activate extracellular-signal regulated kinases (ERK1/2) upon stimulation with its natural ligand, glutamate, or other agonists (Ferraguti et al., 1999; Thandi et al., 2002). It is well known that the Ras-Raf-MEK-ERK module of the MAPK signaling cascade partially controls cell proliferation; alterations in the balance of the MAPK signals could contribute to loss of cellular growth control (Cohen et al., 2002; Kolch, 2000; Mercer and Pritchard, 2003; Peyssonnaux and Eychene, 2001). Normally, cells require extracellular mitogenic signals to proliferate through this cascade; however, several lines of evidence indicate that the MAPK pathway is constitutively stimulated in human melanomas. For example, activating BRAF mutation (V600E) has been identified in a large percentage of biopsies from human malignant melanoma and nevi and is thought to involve in the constitutive activities of MAPK. This indicates that BRAF activation is an early and critical step in the initiation of melanocytic neoplasia (Calipel et al., 2003; Davies et al., 2002; Karasarides et al., 2004; Kumar et al., 2003; Laud et al., 2003; Pollock et al., 2003b; Uribe et al., 2003; Wan et al., 2004). This idea is further supported by results from additional studies in which activated ERK was inhibited via RNAi of mutated BRAF led to a decrease in tumor cell growth (Hingorani et al., 2003; Karasarides et al., 2004; Satyamoorthy et al., 2003). Also, an inhibitor of the MEK kinase, CI1040 (Sebolt-Leopold et al., 1999), when administered orally, was successful in inhibiting the establishment and maintenance of metastatic melanoma (Collisson et al., 2003). These results provide strong evidence that the Ras-RAF-MEK-ERK pathway plays a critical role in melanocytic tumorigenesis.

In our laboratory, we have generated a transgenic mouse line that displays spontaneous melanoma. These transgenic mice were the classical example of insertional mutagenesis. The transgene, Clone B DNA – a small fragment of genomic sequence that was isolated by our laboratory was able to commit a variety of cells in culture to undergo adipocyte differentiation (Chen et al., 1989; Colon-Teicher et al., 1993). There were five independent founder mice, each possessing a different transgene-integration site. The expected phenotype was obesity, but all the mice were of normal weight. After eight months, raised melanotic lesions were detected in one of the transgenic founders (TG-3), and were confirmed as melanomas by histological means. Subsequent progeny homozygous at the transgene integration/host deleted region showed pigmented lesions as early as one month of age with 100% penetrance. These lesions progressed to overt tumors and invaded nearby tissues as the mice grew to adulthood. Mice heterozygous at the same region showed identical tumor phenotypes and progression as the homozygous mice but at later ages. Animals of the other four transgenic lines remained normal even past 2 years of age (Chen et al., 1996; Zhu et al., 1998).

From genome mapping studies on the TG-3 mouse line, we were able to determine that approximately 70kb of the host sequences on chromosome 10 had been deleted by insertion of the transgene. This region of mouse chromosome 10 is syntenic to human chromosome 6q. A large number of human non-familial malignant melanomas display rearrangements in this same region of chromosome 6 (Thompson et al., 1995; Trent et al., 1990). We identified the deleted 70kb host region to be part of intron 3 of the gene encoding metabotropic glutamate receptor 1 (Grm1). To undeniably demonstrate that Grm1 has a direct etiological role in melanoma development in our model system and to distinguish between causes and consequences of ectopic expression of Grm1 in tumor tissues, we generated a new transgenic line with wild-type mouse Grm1 cDNA under a melanocyte-specific promoter, Dct (dopachrome tautomerase). Pigmented tumors developed in the founder and subsequent progeny of this new transgenic line (line E; Pollock et al., 2003a). These results unequivocally demonstrated that the introduction of Grm1 alone was sufficient to induce melanoma development in vivo.

Identification of aberrant Grm1 expression in melanocytes being the causative agent in melanoma development in TG-3/E directed us to extend our studies to the human form of the disease. Initially, we demonstrated GRM1 expression in seven of 19 melanoma biopsies and 12 of 18 human melanoma cell lines (Pollock et al., 2003a). To date, we have tested over 120 human melanoma biopsies and 25 human melanoma cell lines, and found approximately 40% of theses samples express GRM1 at levels of both RNA and protein (Namkoong et al., 2007). Recently we also investigated GRM1 expression in a custom-designed tissue array with duplicated cores made from 38 melanoma and 15 normal skin biopsies. Positive GRM1 expression was detected in 39.5% (15/38) of melanoma samples while none of the 15 normal skin biopsies showed GRM1 expression. In addition, a meeting report by Funusaka et al. (2006) showed GRM1 expression in 80% (49/61) of melanoma tissue samples consisting of superficial spreading, nodular, lentigo maligna, acral lentiginous and metastatic melanomas. GRM1 expression was detected in 33% (6/18) of common, blue and Spitz nevi. Positive GRM1 was observed in 75% (6/8) human melanoma cell lines and 50% (2/4) of nevus lines while none of normal melanocytes was positive. These additional data strongly suggest that a better understanding on the regulation of GRM1 expression in melanocytes at the molecular level contribute to the design of more effective treatment of this disease.

In our mouse model system, we have shown that cell lines derived from mouse tumors and tumor biopsies were wild type for both NRAS/BRAF at the most common mutation sites (codon 600 for BRAF and codons 12, 13 and 61 for NRAS). Furthermore, we also demonstrated that the aberrantly expressed Grm1 is functional in these mouse cell lines; they responded to stimulation or suppression by agonist or antagonist of Grm1 (also dNmutants or siRNA), while Grm5-antagonist had no effect on Grm1-mediated ERK1/2 activation (Marin et al., 2006). In addition, we also showed that Grm1-mediated ERK1/2 activation in these mouse tumor derived cells was mediated by PKCϵ (Marin et al., 2006). On the basis of these results, we concluded that in some melanomas, activated mutations in BRAF are needed to stimulate MAPK signaling, however, in our system, activated BRAF/NRAS mutations are not required for stimulation of ERKs. Rather the aberrant expression of Grm1 in the mouse tumor-derived cells, upon stimulation by Grm1 ligand/agonist, is sufficient to bring about MAPK activation leading to deregulated cellular proliferation and tumor formation.

Similar experiments were performed with three types of human melanoma cell lines with respect to NRAS and BRAF genotypes. Human melanoma cell lines that were either wild type for both BRAF/NRAS (C8161) or carry the common NRAS Q61R mutation (WM239A) were responsive to stimuli/inhibitors of GRM1 in MAPK signaling cascades as we showed earlier in the mouse system (Namkoong et al., 2007). UACC903, a human melanoma cell line with the activating BRAF mutation (V600E) as predicted, already has a constitutively active MAPK;

no further modulation can be mediated by GRM1 agonist or competitive antagonist (J. Namkoong, unpublished results). However, follow-up studies on UACC903 with GRM1-non-competitive antagonist or an inhibitor of glutamate release showed these cells to be responsive to these GRM1 inhibitors (J. Namkoong, unpublished results). These results suggested that activated GRM1 in human melanoma cells mediated its signals in part through the MAPK cascade, one of the major cell proliferation pathways. Additional supporting evidence that GRM1 is critical in regulating melanoma cell growth was demonstrated by several means. We showed that in the presence of a non-competitive GRM1 antagonist, BAY 36-7620, dominant negative GRM1 mutants, or an inhibitor of glutamate release, Riluzole, melanoma cells were accumulated in the subG1 phase of the cell cycle indicative of apoptotic cells, which was confirmed by the appearance of cleaved PARP [poly (ADP-ribose) polymerase] (Namkoong et al., 2007). Recently another group also reported that another antagonist of GRM1 inhibit the in vitro growth of two human melanoma cell lines (Haas et al., 2007). Taken together, these results suggest that non-competitive antagonists of GRM1 or an inhibitor of glutamate release may be used alone and/or with possible synergistic effects of chemotherapy will likely enhance the existing therapies of melanomas.

Conclusion

Surgical excision of early stages of melanoma remains the most successful treatment for this deadly disease. Once it has progressed to the metastatic stage, it does not respond effectively to current therapeutic means. Onset of melanoma can be contributed by both genetic and epigenetic means. Despite much efforts directed towards understanding this disease, a great deal remains unknown and requires further investigation. Our laboratory has been concentrating on malignant melanoma development using animal models. Our working hypothesis is that the ectopic expression of an otherwise normal neuronal protein (Grm1) in an unnatural cellular environment (melanocytes) triggers pathways leading to changes in the transcription of genes involved in regulation of cell growth and ultimately tumor formation. We have shown that human melanoma cell lines release excess extracellular glutamate and propose that in GRM1-positive human melanoma cells the presence of excess glutamate creates an autocrine loop that promotes cell growth. Very similar circumstances have been suggested previously for the high autocrine activity of hepatocyte-growth factor/scatter factor (the ligand) and its receptor (receptor tyrosine kinase, Met; Otsuka et al., 1998). Complementary data supporting the notion that excess extracellular glutamate in melanoma cells may provide an autocrine loop and enhance cell proliferation includes observations reported by others that antagonists of metabotropic and ionotropic glutamate receptors possess growth suppressive activity towards several types of tumors (Haas et al., 2007; Namkoong et al., 2007; Rzeski et al., 2001; Takano et al., 2001). Finally several investigators put forth an intriguing link between neuro-degenerative diseases and melanoma despite limited empirical evidence. On the basis of the multicenter studies in Australia and the USA, the incidence of melanoma among patients with ALS or Parkinson's disease is approximately 2–3 times higher than that of the general population (Baade et al., 2007; Freedman et al., 2005; Inzelberg and Jankovic, 2007). This observation is found to be in accordance with earlier reports that elevated levels of extracellular glutamate have been detected in a large number of human disorders including glioma, multiple sclerosis, Alzheimer, Parkinson, ALS (Corona et al., 2007; Jacob et al., 2007; Sarchielli et al., 2007; Takano et al., 2001; Ye and Sontheimer, 1999) suggest that the common root of melanoma and Parkinson/ALS may be glutamate. These results put forward the importance of glutamate signaling through metabotropic and ionotropic glutamate receptors and imply the possibility of a new target for therapy including suppression of GRM1 mediated glutamate signaling cascades (Namkoong et al., 2007; Nicoletti et al., 2007).

Acknowledgments

We thank Drs Jin Namkoong, Sean Shin and Jeffrey Martino for helpful discussion. This work has been supported by grants: New Jersey Commission for Cancer Research 07-1064-CCR-E0 (H.J. Lee), NCI RO1CA74077 (S. Chen) and NIEHS ES05022 (S. Chen).

References

Abdel-Malek Z, Suzuki I, Tada A, Im S, Akcali C. The melanocortin-1 receptor and human pigmentation. Ann. N Y Acad. Sci 1999;885:117–133. [PubMed: 10816645]

Abdel-Malek Z, Scott MC, Suzuki I, Tada A, Im S, Lamoreux L, Ito S, Barsh G, Hearing VJ. The melanocortin-1 receptor is a key regulator of human cutaneous pigmentation. Pigment Cell Res 2000;13(Suppl 8):156–162. [PubMed: 11041375]

Allen LF, Lefkowitz RJ, Caron MG, Cotecchia S. G-protein-coupled receptor genes as protooncogenes: constitutively activating mutation of the alpha 1B-adrenergic receptor enhances mitogenesis and tumorigenicity. Proc. Natl Acad. Sci. USA 1991;88:11354–11358. [PubMed: 1662393]

Aramori I, Nakanishi S. Signal transduction and pharmacological characteristics of a metabotropic glutamate receptor, mGluR1, in transfected CHO cells. Neuron 1992;8:757–765. [PubMed: 1314623]

Asanuma K, Wakabayashi H, Hayashi T, Okuyama N, Seto M, Matsumine A, Kusuzaki K, Suzuki K, Uchida A. Thrombin inhibitor, argatroban, prevents tumor cell migration and bone metastasis. Oncology 2004;67:166–173. [PubMed: 15539922]

Asokananthan N, Graham PT, Fink J, Knight DA, Bakker AJ, McWilliam AS, Thompson PJ, Stewart GA. Activation of protease-activated receptor (PAR)-1, PAR-2, and PAR-4 stimulates IL-6, IL-8, and prostaglandin E2 release from human respiratory epithelial cells. J. Immunol 2002;168:3577–3585. [PubMed: 11907122]

Atkinson PJ, Young KW, Ennion SJ, Kew JN, Nahorski SR, Challiss RA. Altered expression of G(q/11alpha) protein shapes mGlu1 and mGlu5 receptor-mediated single cell inositol 1,4,5-trisphosphate and Ca(2+) signaling. Mol. Pharmacol 2006;69:174–184. [PubMed: 16234485]

Baade PD, Fritschi L, Freedman DM. Mortality due to amyotrophic lateral sclerosis and Parkinson's disease among melanoma patients. Neuroepidemiology 2007;28:16–20. [PubMed: 17164565]

Bachmann IM, Straume O, Puntervoll HE, Kalvenes MB, Akslen LA. Importance of P-cadherin, beta-catenin, and Wnt5a/frizzled for progression of melanocytic tumors and prognosis in cutaneous melanoma. Clin. Cancer Res 2005;11:8606–8614. [PubMed: 16361544]

Balkwill F. Cancer and the chemokine network. Nat. Rev. Cancer 2004;4:540–550. [PubMed: 15229479]

Balkwill F, Mantovani A. Inflammation and cancer: back to Virchow? Lancet 2001;357:539–545. [PubMed: 11229684]

Bardeesy N, Wong KK, DePinho RA, Chin L. Animal models of melanoma: recent advances and future prospects. Adv. Cancer Res 2000;79:123–156. [PubMed: 10818679]

Battaglia G, Bruno V, Pisani A, Centonze D, Catania MV, Calabresi P, Nicoletti F. Selective blockade of type-1 metabotropic glutamate receptors induces neuroprotection by enhancing gabaergic transmission. Mol. Cell. Neurosci 2001;17:1071–1083. [PubMed: 11414795]

Ben-Baruch A. Organ selectivity in metastasis: regulation by chemokines and their receptors. Clin. Exp. Metastasis 2008;25:345–356. [PubMed: 17891505]

Berger Y, Bernasconi CC, Juillerat-Jeanneret L. Targeting the endothelin axis in human melanoma: combination of endothelin receptor antagonism and alkylating agents. Exp. Biol. Med. (Maywood) 2006;231:1111–1119. [PubMed: 16741059]

Bertrand HO, Bessis AS, Pin JP, Acher FC. Common and selective molecular determinants involved in metabotopic glutamate receptor agonist activity. J. Med. Chem 2002;45:3171–3183. [PubMed: 12109902]

Bittner M, Meltzer P, Chen Y. Molecular classification of cutaneous malignant melanoma by gene expression profiling. Nature 2000;406:536–540. [PubMed: 10952317]

Bordi F, Ugolini A. Group 1 metabotropic glutamate receptors: implications for brain diseases. Prog. Neurobiol 1999;59:55–79. [PubMed: 10416961]

Box NF, Duffy DL, Chen W, Stark M, Martin NG, Sturm RA, Hayward NK. MC1R genotype modifies risk of melanoma in families segregating CDKN2A mutations. Am. J. Hum. Genet 2001;69:765–773. [PubMed: 11500805]

Bradl M, Klein-Santo A, Porter S, Mintz B. Malignant melanoma in transgenic mice. Proc. Natl Acad. Sci. USA 1991;88:164–168. [PubMed: 1846036]

Bruno V, Battaglia G, Kingston A, O'Neill MJ, Catania MV, Di Grezia R, Nicoletti F. Neuroprotective activity of the potent and selective mGlu1a metabotropic glutamate receptor antagonist, (+_-2-methy1-4 carboxyphenylglycine (LY367385): comparison with LY357366, a broader spectrum antagonist with equal affinity for mGlu1a and mGlu5 receptors. Neuropharmacology 1999;38:199–207. [PubMed: 10218860]

Bunemann M, Frank M, Lohse MJ. Gi protein activation in intact cells involves subunit rearrangement rather than dissociation. Proc. Natl Acad. Sci. USA 2003;100:16077–16082. [PubMed: 14673086]

Busca R, Ballotti R. Cyclic AMP a key messenger in the regulation of skin pigmentation. Pigment Cell Res 2000;13:60–69. [PubMed: 10841026]

Busca R, Abbe P, Mantoux F, Aberdam E, Peyssonnauz C, Ballotti R. Ras mediates the cAMP-dependent activation of extracellular signal-regulated kinases (ERKs) in melanocytes. EMBO J 2000;19:2900–2910. [PubMed: 10856235]

Cabrera-Vera TM, Vanhauwe J, Thomas TO, Medkova M, Preininger A, Mazzoni MR, Hamm HE. Insights into G protein structure, function, and regulation. Endocr. Rev 2003;24:765–781. [PubMed: 14671004]

Calipel A, Lefevre G, Pouponnot C, Mouriaux F, Eychene A, Mascarelli F. Mutation of B-Raf in human choroidal melanoma cells mediates cell proliferation and transformation through the MEK/ERK pathway. J. Biol. Chem 2003;278:42409–42418. [PubMed: 12917419]

Cardones AR, Murakami T, Hwang ST. CXCR4 enhances adhesion of B16 tumor cells to endothelial cells in vitro and in vivo via beta(1) integrin. Cancer Res 2003;63:6751–6757. [PubMed: 14583470]

Chambers AF, Groom AC, MacDonald IC. Dissemination and growth of cancer cells in metastatic sites. Nat. Rev. Cancer 2002;2:563–572. [PubMed: 12154349]

Chen S, Tiecher L, Kazim D, Pollack R, Wise L. DNA commitment of mouse fibroblasts to adipocyte differentiation by DNA transfection. Science 1989;244:582–585. [PubMed: 2470149]

Chen S, Zhu H, Wetzel WJ, Philbert MA. Spontaneous melanocytosis in transgenic mice. J. Invest. Dermatol 1996;106:1145–1150. [PubMed: 8618055]

Cheng Z, Zhang L, Graves E, Xiong Z, Dandekar M, Chen X, Gambhir SS. Small-animal PET of melanocortin 1 receptor expression using a 18F-labeled alpha-melanocyte-stimulating hormone analog. J. Nucl. Med 2007;48:987–994. [PubMed: 17504880]

Chin L, Pomerantz J, Polsky D, Jacobson M, Cohen C, Cordon-Cardo C, Horner JW II, DePinho RA. Cooperative effects of INK4a and ras in melanoma susceptibility in vivo. Genes Dev 1997;11:2822–2834. [PubMed: 9353252]

Choe ES, Wang JQ. Regulation of transcription factor phosphorylation by metabotropic glutamate receptor-associated signaling pathways in rat striatal neurons. Neuroscience 2002;114:557–565. [PubMed: 12220559]

Cohen C, Zavala-Pompa A, Sequeira JH, Shoji M, Sexton DG, Cotsonis G, Cerimele F, Govindarajan B, Macaron N, Arbiser JL. Mitogen-actived protein kinase activation is an early event in melanoma progression. Clin. Cancer Res 2002;8:3728–3733. [PubMed: 12473582]

Collisson E, De A, Suzuki H, Gambhir S, Kolodney M. Treatment of metastatic melanoma with an orally available inhibitor of the Ras-Raf-MAPK cascade. Cancer Res 2003;63:5669–5673. [PubMed: 14522881]

Colon-Teicher L, Wise LS, Martino JJ, Baskin L, Sakoulas G, Pollack R, Chen S. Genomic sequences capable of committing mouse and rat fibroblasts to adipogenesis. Nucleic Acids Res 1993;21:2223–2228. [PubMed: 8502564]

Conn PJ, Pin J-P. Pharmacology and functions of metabotropic glutamate receptors. Annu. Rev. Pharmacol. Toxicol 1997;37:205–237. [PubMed: 9131252]

Corona JC, Tovar-y-Romo LB, Tapia R. Glutamate excitotoxicity and therapeutic targets for amyotrophic lateral sclerosis. Expert Opin. Ther. Targets 2007;11:1415–1428. [PubMed: 18028007]

Coussens LM, Werb Z. Inflammation and cancer. Nature 2002;420:860–867. [PubMed: 12490959]

Crnogorac-Jurcevic T, Efthimiou E, Capelli P. Gene expression profiles of pancreatic cancer and stromal desmoplasia. Oncogene 2001;20:7437–7446. [PubMed: 11704875]

Darmoul D, Gratio V, Devaud H, Lehy T, Laburthe M. Aberrant expression and activation of the thrombin receptor protease-activated receptor-1 induces cell proliferation and motility in human colon cancer cells. Am. J. Pathol 2003;162:1503–1513. [PubMed: 12707033]

Davies H, Bignell GR, Cox C. Mutations of the BRAF gene in human cancer. Nature 2002;417:949–954. [PubMed: 12068308]

De Blasi A, Conn PJ, Pin J, Nicoletti F. Molecular determinants of metabotropic glutamate receptor signaling. Trends Pharmacol. Sci 2001;22:114–120. [PubMed: 11239574]

Deo DD, Bazan NG, Hunt JD. Activation of platelet-activating factor receptor-coupled G alpha q leads to stimulation of Src and focal adhesion kinase via two separate pathways in human umbilical vein endothelial cells. J. Biol. Chem 2004;279:3497–3508. [PubMed: 14617636]

Depasquale I, Thompson WD. Prognosis in human melanoma: PAR-1 expression is superior to other coagulation components and VEGF. Histopathology 2008;52:500–509. [PubMed: 18315603]

Dhami GK, Ferguson SS. Regulation of metabotropic glutamate receptor signaling, desensitization and endocytosis. Pharmacol. Ther 2006;111:260–271. [PubMed: 16574233]

Dissanayake SK, Wade M, Johnson CE. The Wnt5A/protein kinase C pathway mediates motility in melanoma cells via the inhibition of metastasis suppressors and initiation of an epithelial to mesenchymal transition. J. Biol. Chem 2007;282:17259–17271. [PubMed: 17426020]

Dorsam RT, Gutkind JS. G-protein-coupled receptors and cancer. Nat. Rev. Cancer 2007;7:79–94. [PubMed: 17251915]

Dunn KJ, Williams BO, Li Y, Pavan WJ. Neural crest-directed gene transfer demonstrates Wnt1 role in melanocyte expansion and differentiation during mouse development. Proc. Natl Acad. Sci. USA 2000;97:10050–10055. [PubMed: 10963668]

Dunn KJ, Brady M, Ochsenbauer-Jambor C, Snyder S, Incao A, Pavan WJ. WNT1 and WNT3a promote expansion of melanocytes through distinct modes of action. Pigment Cell Res 2005;18:167–180. [PubMed: 15892713]

Even-Ram S, Uziely B, Cohen P, Grisaru-Granovsky S, Maoz M, Ginzburg Y, Reich R, Vlodavsky I, Bar-Shavit R. Thrombin receptor overexpression in malignant and physiological invasion processes. Nat. Med 1998;4:909–914. [PubMed: 9701242]

Even-Ram SC, Maoz M, Pokroy E, Reich R, Katz BZ, Gutwein P, Altevogt P, Bar-Shavit R. Tumor cell invasion is promoted by activation of protease activated receptor-1 in cooperation with the alpha vbeta 5 integrin. J. Biol. Chem 2001;276:10952–10962. [PubMed: 11278329]

Ferraguti F, Baldani-Guerra B, Corsi M, Nakanishi S, Corti C. Activation of extracellular signal-regulated kinase 2 by metabotropic glutamate receptors. Eur. J. Neurosci 1999;11:2073–2082. [PubMed: 10336676]

Ferreira MA, Barcelos LS, Teixeira MM, Bakhle YS, Andrade SP. Tumor growth, angiogenesis and inflammation in mice lacking receptors for platelet activating factor (PAF). Life Sci 2007;81:210–217. [PubMed: 17588613]

Fischer EG, Ruf W, Mueller BM. Tissue factor-initiated thrombin generation activates the signaling thrombin receptor on malignant melanoma cells. Cancer Res 1995;55:1629–1632. [PubMed: 7712465]

Forster R, Ohl L, Henning G. Lessons learned from lymphocytes: CC chemokine receptor-7 involved in lymphogenic metastasis of melanoma. J. Natl Cancer Inst 2001;93:1588–1589. [PubMed: 11698555]

Francesconi A, Duvoisin R. Role of the second and third intracellular loops of metabotropic glutamate receptors in mediatic dual signal transduction activation. J. Biol. Chem 1998;273:5615–5624. [PubMed: 9488690]

Frati C, Marchese C, Fisichella G, Copani A, Nasca MR, Storto M, Nicoletti F. Expression of functional mGlu5 metabotropic glutamate receptors in human melanocytes. J. Cell. Physiol 2000;183:364–372. [PubMed: 10797311]

Freedman DM, Travis LB, Gridley G, Kuncl RW. Amyotrophic lateral sclerosis mortality in 1.9 million US cancer survivors. Neuroepidemiology 2005;25:176–180. [PubMed: 16103728]

Funusaka Y, Harada T, Aiba A, Nishigori C. Expression of metabotropic glutamate receptor 1 and phosphorylated extracellular signal-regulated kinase 1/2 proteins in human melanocytic lesions. Pigment Cell Res 2006;19:656. abstract.

Gerstenblith MR, Goldstein AM, Fargnoli MC, Peris K, Landi MT. Comprehensive evaluation of allele frequency differences of MC1R variants across populations. Hum. Mutat 2007;28:495–505. [PubMed: 17279550]

Goldstein AM, Landi MT, Tsang S, Fraser MC, Munroe DJ, Tucker MA. Association of MC1R variants and risk of melanoma in melanoma-prone families with CDKN2A mutations. Cancer Epidemiol. Biomarkers Prev 2005;14:2208–2212. [PubMed: 16172233]

Goldstein AM, Chan M, Harland M. High-risk melanoma susceptibility genes and pancreatic cancer, neural system tumors, and uveal melanoma across GenoMEL. Cancer Res 2006;66:9818–9828. [PubMed: 17047042]

Goldstein AM, Chan M, Harland M. Features associated with germline CDKN2A mutations: a GenoMEL study of melanoma-prone families from three continents. J. Med. Genet 2007;44:99–106. [PubMed: 16905682]

Goodall J, Martinozzi S, Dexter TJ, Champeval D, Carreira S, Larue L, Goding CR. Brn-2 expression controls melanoma proliferation and is directly regulated by beta-catenin. Mol. Cell. Biol 2004;24:2915–2922. [PubMed: 15024079]

Gupta PB, Kuperwasser C, Brunet JP, Ramaswamy S, Kuo WL, Gray JW, Naber SP, Weinberg RA. The melanocyte differentiation program predisposes to metastasis after neoplastic transformation. Nat. Genet 2005;37:1047–1054. [PubMed: 16142232]

Gutkind JS, Novotny EA, Brann MR, Robbins KC. Muscarinic acetylcholine receptor subtypes as agonist-dependent oncogenes. Proc. Natl Acad. Sci. USA 1991;88:4703–4707. [PubMed: 1905013]

Haas H, Pfragner R, Siegl V, Ingolic E, Heintz E, Schraml E, Schauenstein K. The non-competitive metabotropic glutamate receptor-1 antagonist CPCCOEt inhibits the in vitro growth of human melanoma. Oncol. Rep 2007;17:1399–1404. [PubMed: 17487397]

Hamm HE. The many faces of G protein signaling. J. Biol. Chem 1998;273:669–672. [PubMed: 9422713]

Hermans E, Challiss RA. Structural, signalling and regulatory properties of the group I metabotropic glutamate receptors: prototypic family C G-protein-coupled receptors. Biochem. J 2001;359:465–484. [PubMed: 11672421]

Hingorani SR, Jacobetz MA, Robertson GP, Herlyn M, Tuveson DA. Suppression of BRAF(V599E) in human melanoma abrogates transformation. Cancer Res 2003;63:5198–5202. [PubMed: 14500344]

Hsu MY, Meier FE, Nesbit M, Hsu JY, Van Belle P, Elder DE, Herlyn M. E-cadherin expression in melanoma cells restores keratinocyte-mediated growth control and down-regulates expression of invasion-related adhesion receptors. Am. J. Pathol 2000;156:1515–1525. [PubMed: 10793063]

Iacovelli L, Salvatore L, Capobianco L. Role of G protein-coupled receptor kinase 4 and beta-arrestin 1 in agonist-stimulated metabotropic glutamate receptor 1 internalization and activation of mitogen-activated protein kinases. J. Biol. Chem 2003;278:12433–12442. [PubMed: 12519791]

Ihara M, Ishikawa K, Fukuroda T, Saeki T, Funabashi K, Fukami T, Suda H, Yano M. In vitro biological profile of a highly potent novel endothelin (ET) antagonist BQ-123 selective for the ETA receptor. J. Cardiovasc. Pharmacol 1992a;20(Suppl 12):S11–S14. [PubMed: 1282942]

Ihara M, Noguchi K, Saeki T, Fukuroda T, Tsuchida S, Kimura S, Fukami T, Ishikawa K, Nishikibe M, Yano M. Biological profiles of highly potent novel endothelin antagonists selective for the ETA receptor. Life Sci 1992b;50:247–255. [PubMed: 1310132]

Ikeya M, Lee SM, Johnson JE, McMahon AP, Takada S. Wnt signalling required for expansion of neural crest and CNS progenitors. Nature 1997;389:966–970. [PubMed: 9353119]

Im SY, Ko HM, Kim JW. Augmentation of tumor metastasis by platelet-activating factor. Cancer Res 1996;56:2662–2665. [PubMed: 8653713]

Inoue A, Yanagisawa M, Kimura S, Kasuya Y, Miyauchi T, Goto K, Masaki T. The human endothelin family: three structurally and pharmacologically distinct isopeptides predicted by three separate genes. Proc. Natl Acad. Sci. USA 1989;86:2863–2867. [PubMed: 2649896]

Inzelberg R, Jankovic J. Are Parkinson disease patients protected from some but not all cancers? Neurology 2007;69:1542–1550. [PubMed: 17699801]

Iozzo RV, Eichstetter I, Danielson KG. Aberrant expression of the growth factor Wnt-5A in human malignancy. Cancer Res 1995;55:3495–3499. [PubMed: 7627953]

Ishii S, Shimizu T. Platelet-activating factor (PAF) receptor and genetically engineered PAF receptor mutant mice. Prog. Lipid Res 2000;39:41–82. [PubMed: 10729607]

Ishii S, Nagase T, Tashiro F, Ikuta K, Sato S, Waga I, Kume K, Miyazaki J, Shimizu T. Bronchial hyperreactivity, increased endotoxin lethality and melanocytic tumorigenesis in transgenic mice overexpressing platelet-activating factor receptor. EMBO J 1997;16:133–142. [PubMed: 9009274]

Ishikawa K, Ihara M, Noguchi K. Biochemical and pharmacological profile of a potent and selective endothelin B-receptor antagonist, BQ-788. Proc. Natl Acad. Sci. USA 1994;91:4892–4896. [PubMed: 8197152]

Itoh Y, Yanagisawa M, Ohkubo S. Cloning and sequence analysis of cDNA encoding the precursor of a human endothelium-derived vasoconstrictor peptide, endothelin: identity of human and porcine endothelin. FEBS Lett 1988;231:440–444. [PubMed: 3282927]

Itoh K, Brott BK, Bae GU, Ratcliffe MJ, Sokol SY. Nuclear localization is required for Dishevelled function in Wnt/beta-catenin signaling. J. Biol 2005;4:3. [PubMed: 15720724]

Iwamoto T, Takahashi M, Ito M, Hamatani K, Ohbayashi M, Wajjwalku W, Isobe K, Nakashima I. Aberrant melanogenesis and melanocytic tumour development in transgenic mice that carry a metallothionein/ret fusion gene. EMBO J 1991;10:3167–3175. [PubMed: 1915289]

Jacob CP, Koutsilieri E, Bartl J, Neuen-Jacob E, Arzberger T, Zander N, Ravid R, Roggendorf W, Riederer P, Grunblatt E. Alterations in expression of glutamatergic transporters and receptors in sporadic Alzheimer's disease. J. Alzheimers Dis 2007;11:97–116. [PubMed: 17361039]

Jamal S, Schneider RJ. UV-induction of keratinocyte endothelin-1 downregulates E-cadherin in melanocytes and melanoma cells. J. Clin. Invest 2002;110:443–452. [PubMed: 12189238]

Julius D, Livelli TJ, Jessell TM, Axel R. Extopic expresion of the serotonin 1c receptor and the triggering of malignant transformation. Science 1989;244:1057–1062. [PubMed: 2727693]

Kadekaro AL, Kavanagh RJ, Wakamatsu K, Ito S, Pipitone MA, Abdel-Malek ZA. Cutaneous photobiology. The melanocyte vs. the sun: who will win the final round? Pigment Cell Res 2003;16:434–447. [PubMed: 12950718]

Kadekaro AL, Kavanagh R, Kanto H. alpha-Melanocortin and endothelin-1 activate antiapoptotic pathways and reduce DNA damage in human melanocytes. Cancer Res 2005a;65:4292–4299. [PubMed: 15899821]

Kadekaro AL, Kavanagh R, Kanto H. alpha-Melanocortin and endothelin-1 activate antiapoptotic pathways and reduce DNA damage in human melanocytes. Cancer Res 2005b;65:4292–4299. [PubMed: 15899821]

Kakinuma T, Hwang ST. Chemokines, chemokine receptors, and cancer metastasis. J. Leukoc. Biol 2006;79:639–651. [PubMed: 16478915]

Karasarides M, Chiloeches A, Hayward R. B-RAF is a therapeutic target in melanoma. Oncogene 2004;23:6292–6298. [PubMed: 15208680]

Kato M, Takahashi M, Akhand AA, Liu W, Dai Y, Shimizu S, Iwamoto T, Suzuki H, Nakashima I. Transgenic mouse model for skin malignant melanoma. Oncogene 1998;17:1885–1888. [PubMed: 9778055]

Kawada K, Sonoshita M, Sakashita H, Takabayashi A, Yamaoka Y, Manabe T, Inaba K, Minato N, Oshima M, Taketo MM. Pivotal role of CXCR3 in melanoma cell metastasis to lymph nodes. Cancer Res 2004;64:4010–4017. [PubMed: 15173015]

Kedzierski RM, Yanagisawa M. Endothelin system: the double-edged sword in health and disease. Annu. Rev. Pharmacol. Toxicol 2001;41:851–876. [PubMed: 11264479]

Kefford R, Beith JM, Van Hazel GA. A phase II study of bosentan, a dual endothelin receptor antagonist, as monotherapy in patients with stage IV metastatic melanoma. Invest. New Drugs 2007;25:247–252. [PubMed: 17021960]

Kennedy C, ter Huurne J, Berkhout M, Gruis N, Bastiaens M, Bergman W, Willemze R, Bavinck JN. Melanocortin 1 receptor (MC1R) gene variants are associated with an increased risk for cutaneous melanoma which is largely independent of skin type and hair color. J. Invest. Dermatol 2001;117:294–300. [PubMed: 11511307]

Kim M, Gans JD, Nogueira C. Comparative oncogenomics identifies NEDD9 as a melanoma metastasis gene. Cell 2006;125:1269–1281. [PubMed: 16814714]

Kingston AE, Lowndes J, Evans N, Clark B, Tomlinson R, Burnett JP, Mayne NG, Cockerham SL, Lodge D. Sulphur-containing amino acids are agonists for group 1 metabotropic receptors expressed in clonal RGT cell lines. Neuro-pharmacology 1998;37:277–287.

Kingston AE, O'Neill MJ, Bond A. Neuroprotective actions of novel and potent ligands of group I and group II metabotropic glutamate receptors. Ann. N Y Acad. Sci 1999;890:438–449. [PubMed: 10668448]

Klein-Szanto AJ, Silvers WK, Mintz B. Ultraviolet radiation-induced malignant skin melanoma in melanoma- susceptible transgenic mice. Cancer Res 1994;54:4569–4572. [PubMed: 8062242]

Kolch W. Meaningful relationships: the regulation of the Ras/Raf/MEK/ERK pathway by protein interactions. Biochem. J 2000;351:289–305. [PubMed: 11023813]

Krimpenfort P, Quon KC, Mooi WJ, Loonstra A, Berns A. Loss of p16Ink4a confers susceptibility to metastatic melanoma in mice. Nature 2001;413:83–86. [PubMed: 11544530]

Kumar R, Angelini S, Czene K, Sauroja I, Hahka-Kemppinen M, Pyrhonen S, Hemminki K. BRAF mutations in metastatic melanoma: a possible association with clinical outcome. Clin. Cancer Res 2003;9:3362–3368. [PubMed: 12960123]

Lahav R. Endothelin receptor B is required for the expansion of melanocyte precursors and malignant melanoma. Int. J. Dev. Biol 2005;49:173–180. [PubMed: 15906230]

Lahav R, Heffner G, Patterson PH. An endothelin receptor B antagonist inhibits growth and induces cell death in human melanoma cells in vitro and in vivo. Proc. Natl Acad. Sci. USA 1999;96:11496–11500. [PubMed: 10500205]

Lahav R, Suva ML, Rimoldi D, Patterson PH, Stamenkovic I. Endothelin receptor B inhibition triggers apoptosis and enhances angiogenesis in melanomas. Cancer Res 2004;64:8945–8953. [PubMed: 15604257]

Landi MT, Bauer J, Pfeiffer RM, Elder DE, Hulley B, Minghetti P, Calista D, Kanetsky PA, Pinkel D, Bastian BC. MC1R germline variants confer risk for BRAF-mutant melanoma. Science 2006;313:521–522. [PubMed: 16809487]

Laud K, Kannengiesser C, Avril MF, Chompret A, StoppaLyonnet D, Desjardins L, Eychene A, Demenais F, Lenoir GM, Bressac-de Paillerets B. BRAF as a melanoma susceptibility candidate gene? Cancer Res 2003;63:3061–3065. [PubMed: 12810628]

Li S, Huang S, Peng SB. Overexpression of G protein-coupled receptors in cancer cells: involvement in tumor progression. Int. J. Oncol 2005;27:1329–1339. [PubMed: 16211229]

Lin YC, You L, Xu Z. Wnt inhibitory factor-1 gene transfer inhibits melanoma cell growth. Hum. Gene Ther 2007;18:379–386. [PubMed: 17472570]

Littman L, Chase LA, Renzi M, Garlin AB, Koerner JF, Johnson RL, Robinson MB. Effects of quisqualic acid analogs on metabotropic glutamate receptors coupled to phosphoinositide hydrolysis in rat hippocampus. Neuropharmacology 1995;34:829–841. [PubMed: 8532164]

Lundstrom K. Latest development in drug discovery on G protein-coupled receptors. Curr. Protein Pept. Sci 2006;7:465–470. [PubMed: 17073697]

Madamanchi NR, Li S, Patterson C, Runge MS. Thrombin regulates vascular smooth muscle cell growth and heat shock proteins via the JAK-STAT pathway. J. Biol. Chem 2001;276:18915–18924. [PubMed: 11278437]

Mantovani A. Cancer: inflammation by remote control. Nature 2005;435:752–753. [PubMed: 15944689]

Marin Y, Chen S. Involvement of metabotropic glutamate receptor 1, a G-protein-coupled receptor, in melanoma development. J. Mol. Med 2004;82:735–749. [PubMed: 15322701]

Marin YE, Namkoong J, Shin SS, Raines J, Degenhardt K, White E, Chen S. Grm5 expression is not required for the oncogenic role of Grm1 in melanocytes. Neuropharmacology 2005;49(Suppl 1):70–79. [PubMed: 16040064]

Marin YE, Namkoong J, Cohen-Solal K, Shin SS, Martino JJ, Oka M, Chen S. Stimulation of oncogenic metabotropic glutamate receptor 1 in melanoma cells activates ERK1/2 via PKCepsilon. Cell Signal 2006;18:1279–1286. [PubMed: 16305822]

Marinissen MJ, Gutkind JS. G-protein-coupled receptors and signaling networks: emerging paradigms. Trends Pharmacol. Sci 2001;22:368–376. [PubMed: 11431032]

Massi D, Naldini A, Ardinghi C. Expression of protease-activated receptors 1 and 2 in melanocytic nevi and malignant melanoma. Hum. Pathol 2005;36:676–685. [PubMed: 16021575]

Matichard E, Verpillat P, Meziani R. Melanocortin 1 receptor (MC1R) gene variants may increase the risk of melanoma in France independently of clinical risk factors and UV exposure. J. Med. Genet 2004;41:e13. [PubMed: 14757863]

Melnikova V, Bar-Eli M. Inflammation and melanoma growth and metastasis: the role of platelet-activating factor (PAF) and its receptor. Cancer Metastasis Rev 2007;26:359–371. [PubMed: 17721743]

Melnikova VO, Mourad-Zeidan AA, Lev DC, Bar-Eli M. Platelet-activating factor mediates MMP-2 expression and activation via phosphorylation of cAMP-response element-binding protein and contributes to melanoma metastasis. J. Biol. Chem 2006;281:2911–2922. [PubMed: 16306050]

Mercer KE, Pritchard CA. Raf proteins and cancer: B-Raf is identified a s a mutational target. Biochim. Biophys. Acta 2003;1653:25–40. [PubMed: 12781369]

Mills GB, Moolenaar WH. The emerging role of lysophosphatidic acid in cancer. Nat. Rev. Cancer 2003;3:582–591. [PubMed: 12894246]

Mintz B, Silvers WK, Klein-Szanto AJP. Histopatho-genesis of malignant skin melanoma induced in genetically susceptible transgenic mice. Proc. Natl Acad. Sci. USA 1993;90:8822–8826. [PubMed: 8415614]

Murakami T, Maki W, Cardones AR, Fang H, Tun Kyi A, Nestle FO, Hwang ST. Expression of CXC chemokine receptor-4 enhances the pulmonary metastatic potential of murine B16 melanoma cells. Cancer Res 2002;62:7328–7334. [PubMed: 12499276]

Murakami T, Cardones AR, Finkelstein SE, Restifo NP, Klaunberg BA, Nestle FO, Castillo SS, Dennis PA, Hwang ST. Immune evasion by murine melanoma mediated through CC chemokine receptor-10. J. Exp. Med 2003;198:1337–1347. [PubMed: 14581607]

Murakami T, Cardones AR, Hwang ST. Chemokine receptors and melanoma metastasis. J. Dermatol. Sci 2004;36:71–78. [PubMed: 15519136]

Murphy PM, Baggiolini M, Charo IF, Hebert CA, Horuk R, Matsushima K, Miller LH, Oppenheim JJ, Power CA. International union of pharmacology. XXII. Nomenclature for chemokine receptors. Pharmacol. Rev 2000;52:145–176. [PubMed: 10699158]

Namkoong J, Shin SS, Lee HJ, Marin YE, Wall BA, Goydos JS, Chen S. Metabotropic glutamate receptor 1 and glutamate signaling in human melanoma. Cancer Res 2007;67:2298–2305. [PubMed: 17332361]

Neves SR, Ram PT, Iyengar R. G protein pathways. Science 2002;296:1636–1639. [PubMed: 12040175]

Newton RA, Cook AL, Roberts DW, Leonard JH, Sturm RA. Post-transcriptional regulation of melanin biosynthetic enzymes by cAMP and resveratrol in human melanocytes. J. Invest. Dermatol 2007a; 127:2216–2227. [PubMed: 17460731]

Newton RA, Roberts DW, Leonard JH, Sturm RA. Human melanocytes expressing MC1R variant alleles show impaired activation of multiple signaling pathways. Peptides 2007b;28:2387–2396. [PubMed: 18006116]

Nicoletti F, Arcella A, Iacovelli L, Battaglia G, Giangaspero F, Melchiorri D. Metabotropic glutamate receptors: new targets for the control of tumor growth? Trends Pharmacol. Sci 2007;28:206–213. [PubMed: 17433452]

Nierodzik ML, Kajumo F, Karpatkin S. Effect of thrombin treatment of tumor cells on adhesion of tumor cells to platelets in vitro and tumor metastasis in vivo. Cancer Res 1992;52:3267–3272. [PubMed: 1596884]

Nierodzik ML, Chen K, Takeshita K, Li JJ, Huang YQ, Feng XS, D'Andrea MR, Andrade-Gordon P, Karpatkin S. Protease-activated receptor 1 (PAR-1) is required and rate-limiting for thrombin-enhanced experimental pulmonary metastasis. Blood 1998;92:3694–3700. [PubMed: 9808563]

Noonan FP, Dudek J, Merlino G, De Fabo EC. Animal models of melanoma: an HGF/SF transgenic mouse model may facilitate experimental access to UV initiating events. Pigment Cell Res 2003;16:16–25. [PubMed: 12519121]

Ohashi H, Maruyama T, Higashi-Matsumoto H, Nomoto T, Nishimura S, Takeuchi Y. A novel binding assay for metabotropic glutamate receptors using [3H] L-quisqualic acid and recombinant receptors. Z. Naturforsch. [C] 2002;57:348–355.

Olson DJ, Gibo DM, Saggers G, Debinski W, Kumar R. Reversion of uroepithelial cell tumorigenesis by the ectopic expression of human wnt-5a. Cell Growth Differ 1997;8:417–423. [PubMed: 9101087]

Omholt K, Platz A, Ringborg U, Hansson J. Cytoplasmic and nuclear accumulation of beta-catenin is rarely caused by CTNNB1 exon 3 mutations in cutaneous malignant melanoma. Int. J. Cancer 2001;92:839–842. [PubMed: 11351304]

Otsuka T, Takayama H, Sharp R. c-Met autocrine activation induces development of malignant melanoma and acquisition of the metastatic phenotype. Cancer Res 1998;58:5157–5167. [PubMed: 9823327]

Palmer JS, Duffy DL, Box NF, Aitken JF, O'Gorman LE, Green AC, Hayward NK, Martin NG, Sturm RA. Melanocortin-1 receptor polymorphisms and risk of melanoma: is the association explained solely by pigmentation phenotype? Am. J. Hum. Genet 2000;66:176–186. [PubMed: 10631149]

Peyssonnaux C, Eychene A. The Raf/MEK/ERK pathway: new concepts of activation. Biol. Cell 2001;93:53–62. [PubMed: 11730323]

Pierce KL, Premont RT, Lefkowitz RJ. Seven-transmembrane receptors. Nat. Rev. Mol. Cell Biol 2002;3:639–650. [PubMed: 12209124]

Pikarsky E, Porat RM, Stein I, Abramovitch R, Amit S, Kasem S, Gutkovich-Pyest E, Urieli-Shoval S, Galun E, Ben-Neriah Y. NF-kappaB functions as a tumour promoter in inflammation-associated cancer. Nature 2004;431:461–466. [PubMed: 15329734]

Pin J-P, Duvoisin R. The metabotropic glutamate receptors: structure and functions. Neuropharmcology 1995;34:1–26.

Pisani A, Calabresi P, Centonze D, Bernardi G. Enhancement of NMDA responses by group I metabotropic glutamate receptor activation in striatal neurones. Br. J. Pharmacol 1997;120:1007–1014. [PubMed: 9134210]

Polakis P. Wnt signaling and cancer. Genes Dev 2000;14:1837–1851. [PubMed: 10921899]

Pollock PM, Cohen-Solal K, Sood R. Melanoma mouse model implicates metabotropic glutamate signaling in melanocytic neoplasia. Nat. Genet 2003a;34:108–112. [PubMed: 12704387]

Pollock P, Harper U, Hansen K. High frequency of BRAF mutations in nevi. Nat. Genet 2003b;33:19–20. [PubMed: 12447372]

Powell MB, Gause PR, Hyman P, Gregus J, Lluria-Prevatt M, Nagle R, Bowden GT. Induction of melanoma in TPras transgenic mice. Carcinogenesis 1999;20:1747–1753. [PubMed: 10469620]

Prescott SM, Zimmerman GA, Stafforini DM, McIntyre TM. Platelet-activating factor and related lipid mediators. Annu. Rev. Biochem 2000;69:419–445. [PubMed: 10966465]

Rees JL. Genetics of hair and skin color. Annu. Rev. Genet 2003;37:67–90. [PubMed: 14616056]

Reya T, Clevers H. Wnt signalling in stem cells and cancer. Nature 2005;434:843–850. [PubMed: 15829953]

Richard DE, Vouret-Craviari V, Pouyssegur J. Angiogenesis and G-protein-coupled receptors: signals that bridge the gap. Oncogene 2001;20:1556–1562. [PubMed: 11313902]

Riedel G, Platt B, Micheau J. Glutamate receptor function in learning and memory. Behav. Brain Res 2003;140:1–47. [PubMed: 12644276]

Rimm DL, Caca K, Hu G, Harrison FB, Fearon ER. Frequent nuclear/cytoplasmic localization of beta-catenin without exon 3 mutations in malignant melanoma. Am. J. Pathol 1999;154:325–329. [PubMed: 10027390]

Robert EG, Hunt JD. Lipid messengers as targets for antiangiogenic therapy. Curr. Pharm. Des 2001;7:1615–1626. [PubMed: 11562302]

Roberts DW, Newton RA, Beaumont KA, Helen Leonard J, Sturm RA. Quantitative analysis of MC1R gene expression in human skin cell cultures. Pigment Cell Res 2006;19:76–89. [PubMed: 16420249]

Roberts DW, Newton RA, Sturm RA. MC1R expression in skin: is it confined to melanocytes? J. Invest. Dermatol 2007;127:2472–2473. [PubMed: 17508022]

Roberts DW, Newton RA, Leonard JH, Sturm RA. Melanocytes expressing MC1R polymorphisms associated with red hair color have altered MSH-ligand activated pigmentary responses in coculture with keratinocytes. J. Cell. Physiol 2008;215:344–355. [PubMed: 17960564]

Robledo MM, Bartolome RA, Longo N, Rodriguez-Frade JM, Mellado M, Longo I, van Muijen GN, Sanchez-Mateos P, Teixido J. Expression of functional chemokine receptors CXCR3 and CXCR4 on human melanoma cells. J. Biol. Chem 2001;276:45098–45105. [PubMed: 11571298]

Rot A, von Andrian UH. Chemokines in innate and adaptive host defense: basic chemokinese grammar for immune cells. Annu. Rev. Immunol 2004;22:891–928. [PubMed: 15032599]

Rozengurt E, Guha S, Sinnett-Smith J. Gastrointestinal peptide signalling in health and disease. Eur. J. Surg. Suppl 2002;587:23–38. [PubMed: 16144198]

Ruf W, Dorfleutner A, Riewald M. Specificity of coagulation factor signaling. J. Thromb. Haemost 2003;1:1495–1503. [PubMed: 12871285]

Rzeski W, Turski L, Ikonomidou C. Glutamate antagonists limit tumor growth. Proc. Natl Acad. Sci. USA 2001;98:6372–6377. [PubMed: 11331750]

Sarchielli P, Di Filippo M, Candeliere A, Chiasserini D, Mattioni A, Tenaglia S, Bonucci M, Calabresi P. Expression of ionotropic glutamate receptor GLUR3 and effects of glutamate on MBP- and MOG-specific lymphocyte activation and chemotactic migration in multiple sclerosis patients. J. Neuroimmunol 2007;188:146–158. [PubMed: 17628700]

Sato S, Kume K, Ito C, Ishii S, Shimizu T. Accelerated proliferation of epidermal keratinocytes by the transgenic expression of the platelet-activating factor receptor. Arch. Dermatol. Res 1999;291:614–621. [PubMed: 10638335]

Satyamoorthy K, Li G, Gerrero MR, Brose MS, Volpe P, Weber BL, Van Belle P, Elder DE, Herlyn M. Constitutive mitogen-activated protein kinase activation in melanoma is mediated by both BRAF mutations and autocrine growth factor stimulation. Cancer Res 2003;63:756–759. [PubMed: 12591721]

Scala S, Giuliano P, Ascierto PA. Human melanoma metastases express functional CXCR4. Clin. Cancer Res 2006;12:2427–2433. [PubMed: 16638848]

Schlyer S, Horuk R. I want a new drug: G-protein-coupled receptors in drug development. Drug Discov. Today 2006;11:481–493. [PubMed: 16713899]

Sebolt-Leopold JS, Dudley DT, Herrera R. Blockade of the MAP kinase pathway suppresses growth of colon tumors in vivo. Nat. Med 1999;5:810–816. comment. [PubMed: 10395327]

Seidl H, Richtig E, Tilz H, Stefan M, Schmidbauer U, Asslaber M, Zatloukal K, Herlyn M, Schaider H. Profiles of chemokine receptors in melanocytic lesions: de novo expression of CXCR6 in melanoma. Hum. Pathol 2007;38:768–780. [PubMed: 17306330]

Simonetti O, Goteri G, Lucarini G, Filosa A, Pieramici T, Rubini C, Biagini G, Offidani A. Potential role of CCL27 and CCR10 expression in melanoma progression and immune escape. Eur. J. Cancer 2006;42:1181–1187. [PubMed: 16644201]

Skinner PJ, Vierra-Green CA, Clark HB, Zoghbi HY, Orr HT. Altered trafficking of membrane proteins in purkinje cells of SCA1 transgenic mice. Am. J. Pathol 2001;159:905–913. [PubMed: 11549583]

Smith AG, Luk N, Newton RA, Roberts DW, Sturm RA, Muscat GE. Melanocortin-1 receptor signalling markedly induces the expression of the NR4A nuclear receptor subgroup in melanocytic cells. J. Biol. Chem 2008;283:12564–12570. [PubMed: 18292087]

Soufir N, Meziani R, Lacapere JJ. Association between endothelin receptor B nonsynonymous variants and melanoma risk. J. Natl Cancer Inst 2005;97:1297–1301. [PubMed: 16145050]

Soufir N, Ollivaud L, Bertrand G. A French CDK4-positive melanoma family with a co-inherited EDNRB mutation. J. Dermatol. Sci 2007;46:61–64. [PubMed: 17223014]

Stafforini DM, McIntyre TM, Zimmerman GA, Prescott SM. Platelet-activating factor, a pleiotrophic mediator of physiological and pathological processes. Crit. Rev. Clin. Lab. Sci 2003;40:643–672. [PubMed: 14708958]

Storto M, de Grazia U, Knopfel T, Canonico PL, Copani A, Richelmi P, Nicoletti F, Vairetti M. Selective blockade of mGlu5 metabotropic glutamate receptors protects rat hepatocytes against hypoxic damage. Hepatology 2000;31:649–655. [PubMed: 10706555]

Suzuki I, Im S, Tada A, Scott C, Akcali C, Davis MB, Barsh G, Hearing V, Abdel-Malek Z. Participation of the melanocortin-1 receptor in the UV control of pigmentation. J. Investig. Dermatol. Symp. Proc 1999;4:29–34.

Takano T, Lin JH, Arcuino G, Gao Q, Yang J, Nedergaard M. Glutamate release promotes growth of malignant gliomas. Nat. Med 2001;7:1010–1015. [PubMed: 11533703]

Takayama H, LaRochelle WJ, Sharp R, Otsuka T, Kriebel P, Anver M, Aaronson SA, Merlino G. Diverse tumorigenesis associated with aberrant development in mice overexpressing hepatocyte growth factor/scatter factor. Proc. Natl Acad. Sci. USA 1997;94:701–706. [PubMed: 9012848]

Takeuchi H, Fujimoto A, Tanaka M, Yamano T, Hsueh E, Hoon DS. CCL21 chemokine regulates chemokine receptor CCR7 bearing malignant melanoma cells. Clin. Cancer Res 2004;10:2351–2358. [PubMed: 15073111]

Tellez C, Bar-Eli M. Role and regulation of the thrombin receptor (PAR-1) in human melanoma. Oncogene 2003;22:3130–3137. [PubMed: 12789289]

Tellez C, McCarty M, Ruiz M, Bar-Eli M. Loss of activator protein-2alpha results in overexpression of protease-activated receptor-1 and correlates with the malignant phenotype of human melanoma. J. Biol. Chem 2003;278:46632–46642. [PubMed: 12975361]

Tellez CS, Davis DW, Prieto VG, Gershenwald JE, Johnson MM, McCarty MF, Bar-Eli M. Quantitative analysis of melanocytic tissue array reveals inverse correlation between activator protein-2alpha and protease-activated receptor-1 expression during melanoma progression. J. Invest. Dermatol 2007;127:387–393. [PubMed: 16946713]

Thandi S, Blank JL, Challiss RA. Group-I metabotropic glutamate receptors, mGlu1a and mGlu5a, couple to extracellular signal-regulated kinase (ERK) activation via distinct, but overlapping, signalling pathways. J. Neurochem 2002;83:1139–1153. [PubMed: 12437585]

Thirumaran RK, Thoelke A, Ugurel S, Hemminki K, Schadendorf D, Kumar R. Re: association between endothelin receptor B nonsynonymous variants and melanoma risk. J. Natl Cancer Inst 2006;98:1252–1253. [PubMed: 16954478]

Thompson FH, Emerson J, Olson S. Cytogenetics of 158 patients with regional or disseminated melanoma. Subset analysis of near-diploid and simple karyotypes. Cancer Genet. Cytogenet 1995;83:93–104. [PubMed: 7553595]

Topol L, Jiang X, Choi H, Garrett-Beal L, Carolan PJ, Yang Y. Wnt-5a inhibits the canonical Wnt pathway by promoting GSK-3-independent beta-catenin degradation. J. Cell Biol 2003;162:899–908. [PubMed: 12952940]

Trent J, Stanbridge E, McBride H, Meese E, Casey G, Araujo D, Witkowski C, Nagle R. Tumorigenicity in human melanoma cell lines controlled by introduction of human chromosome 6. Science 1990;247:568–571. [PubMed: 2300817]

Uribe P, Wistuba II, Gonzalez S. BRAF mutation: a frequent event in benign, atypical, and malignant melanocytic lesions of the skin. Am. J. Dermatopathol 2003;25:365–370. [PubMed: 14501284]

Valenti O, Conn PJ, Marino MJ. Distinct physiological roles of the Gq-coupled metabotropic glutamate receptors co-expressed in the same neuronal populations. J. Cell. Physiol 2002;191:125–137. [PubMed: 12064455]

Valverde P, Healy E, Sikkink S, Haldane F, Thody AJ, Carothers A, Jackson IJ, Rees JL. The Asp84Glu variant of the melanocortin 1 receptor (MC1R) is associated with melanoma. Hum. Mol. Genet 1996;5:1663–1666. [PubMed: 8894704]

Wan PT, Garnett MJ, Roe SM. Mechanism of activation of the RAF-ERK signaling pathway by oncogenic mutations of B-RAF. Cell 2004;116:855–867. see comment. [PubMed: 15035987]

Weeraratna AT. A Wnt-er wonderland – the complexity of Wnt signaling in melanoma. Cancer Metastasis Rev 2005;24:237–250. [PubMed: 15986134]

Weeraratna AT, Jiang Y, Hostetter G, Rosenblatt K, Duray P, Bittner M, Trent JM. Wnt5a signaling directly affects cell motility and invasion of metastatic melanoma. Cancer Cell 2002;1:279–288. [PubMed: 12086864]

Ye ZC, Sontheimer H. Glioma cells release excitotoxic concentrations of glutamate. Cancer Res 1999;59:4383–4391. [PubMed: 10485487]

Yin YJ, Salah Z, Maoz M, Ram SC, Ochayon S, Neufeld G, Katzav S, Bar-Shavit R. Oncogenic transformation induces tumor angiogenesis: a role for PAR1 activation. FASEB J 2003;17:163–174. [PubMed: 12554695]

Young D, Waitches G, Birchmeier C, Fasano O, Wigler M. Isolation and characterization of a new cellular oncogene encoding a protein with multiple potential transmembrane domains. Cell 1986;45:711–719. [PubMed: 3708691]

Zain J, Huang YQ, Feng X, Nierodzik ML, Li JJ, Karpatkin S. Concentration-dependent dual effect of thrombin on impaired growth/apoptosis or mitogenesis in tumor cells. Blood 2000;95:3133–3138. [PubMed: 10807779]

Zhu H, Reuhl K, Zhang X, Botha R, Ryan K, Wei J, Chen S. Development of heritable melanoma in transgenic mice. J. Invest. Dermatol 1998;110:247–252. [PubMed: 9506443]

Zimmerman GA, McIntyre TM, Prescott SM, Stafforini DM. The platelet-activating factor signaling system and its regulators in syndromes of inflammation and thrombosis. Crit. Care Med 2002;30:S294–S301. [PubMed: 12004251]