# Hard Times for Oncogenic BRAF-Expressing Melanoma Cells

Miles D. Houslay[1,*]
[1]Molecular Pharmacology Group, Institute of Neuroscience and Psychology, CMVLS, Wolfson Link and Davidson Buildings, University of Glasgow, Glasgow G12 8QQ, Scotland, UK
*Correspondence: miles.houslay@glasgow.ac.uk
DOI 10.1016/j.ccr.2011.01.004

**In this issue of *Cancer Cell*, Arozarena et al. describe that the oncogenic BRAF Val600Glu mutant, which occurs in about half of melanomas, downregulates the cGMP-hydrolysing phosphodiesterase PDE5A in melanoma cells through the ERK-MAPK cascade coupled to the POU-domain transcription factor BRN2, thereby increasing intracellular cGMP levels and promoting invasiveness.**

Melanocytes provide critical protection of skin from ultraviolet radiation. However, gene mutations can lead to malignant cutaneous melanoma, whose incidence is on the increase. Although surgical removal of the tumor provides an effective first line therapy, progression occurs in around 20% of cases to an aggressive, metastasizing form of the disease with extremely poor patient survival rates. Nearly half of all melanomas carry an activating mutation in BRAF, one of the three forms of the RAF protein kinase family activated by Ras in the ERK protein kinase pathway that promotes cell proliferation. Ninety percent of such BRAF mutations involve a change of the hydrophobic Val600 for a negatively charged Glu, identifying activated Val600Glu-BRAF as a potential therapeutic target (Bollag et al., 2010).

The Val600Glu-BRAF oncogene causes the upregulation of a large cohort of genes in melanoma cells and, intriguingly, the downregulation of a much more restricted set (Packer et al., 2009). Recently, Marais and colleagues (Arozarena et al., 2011) have provided an extremely exciting and novel insight into the critical issue of melanoma cell invasiveness, discovering that it can be elicited by the downregulation of PDE5A (Figure 1), a cyclic nucleotide phosphodiesterase that specifically hydrolyses the second messenger, cGMP (Francis et al., 2009). Active-site directed selective inhibitors of this enzyme are used acutely for the treatment of penile erectile dysfunction (PED) and infantile hypertension (Francis et al., 2009). It has also been suggested that chronic application of PDE5 selective inhibitors may have potential for treating leukemia, colorectal carcinoma, and breast cancer (see, e.g., Zhu et al., 2005), although recent studies have questioned whether PDE5 is the target (Abadi et al., 2010), as well as for treating heart failure (Kass et al., 2007).

Eleven cyclic nucleotide phosphodiesterase (PDE) families provide over 70 distinct proteins that hydrolyze either or both the second messengers, cAMP and cGMP (Conti and Beavo, 2007). The impressive diversity of this super family reflects their ability to provide key nodes for cross-talk between signaling pathways via both distinct regulatory domains and posttranslational modification through phosphorylation, ubiquitination, and SUMOylation (Houslay, 2010). Critically, various PDEs possess targeting sequences for sequestration by scaffold and other proteins into "signalosomes," conferring signal compartmentalization through spatial regulation of cyclic nucleotide degradation (Houslay, 2010).

The *PDE5A* gene locates to human chromosome 4q25-27 and encodes three kinetically identical variants distinguished by distinct N-terminal regions (Francis et al., 2009). The functional role for isoform diversity is unknown. However, their tissue distribution varies considerably, and it is possible that the distinct N-terminal regions are involved in targeting PDE5A isoforms to distinct signaling complexes, as occurs with PDE4 isoforms (Houslay, 2010). PDE5 isoforms exhibit twin GAF domains, which represent one of the largest and most widespread of the small-molecule binding domains found in nature. The GAF A domain binds cGMP and facilitates the PKG/PKA-dependent phosphorylation and activation of PDE5. Structural studies show that the two N-terminal GAF A domains interact with each other and may serve to stabilize a PDE5 dimer. Importantly, however, the PDE5 catalytic unit is the target for various highly selective competitive inhibitors used to treat PED, namely Viagra, Zydena, Cialis, and Levitra. These compounds exert a relaxing effect on the vasculature of the penis; but this occurs only in the presence of an external neuronal or endothelial stimulus that can promote increased nitric oxide generation. Thus, nitric oxide activates guanylyl cyclase, causing cGMP production that is amplified by inhibitor blockade of PDE5-mediated cGMP degradation. Elevated cGMP levels activate PKG, which phosphorylates several substrates in smooth muscle, such as $Ca^{2+}$-activated maxi $K^+$ ($BK_{Ca}$) channels, IRAG ($IP_3$ receptor associated cGMP kinase substrate), and the regulatory myosin-binding subunit of myosin phosphatase, that lead to a reduction in intracellular $Ca^{2+}$ and, consequently, to smooth muscle relaxation.

Marais and colleagues (Arozarena et al., 2011) now uncover a hitherto unforeseen role for PDE5A in controlling the invasiveness of melanoma cells. They demonstrate that oncogenic, activated BRAF acts through MEK and the POU-domain transcription factor BRN2 to downregulate PDE5A. This leads to a rise in cGMP, which causes elevation of intracellular $Ca^{2+}$ levels and a dramatic and consequential increase in melanoma cell invasion that depends upon increased myosin light chain 2 (MLC2) phosphorylation. Thus, enhanced cGMP/$Ca^{2+}$ -regulated actin-myosin contractility

is essential for invasion induced by PDE5A downregulation. This observation was very surprising because, in vascular smooth muscle cells, cGMP elicits relaxation, indicating that the "wiring" associated with cGMP signaling is very different in melanoma cells. They went on to confirm the pivotal role of PDE5A in this process, as ectopic expression of any PDE5A isoform suppressed the invasiveness of Val600-Glu-BRAF-expressing melanoma cells. Furthermore, they found that PDE5A was downregulated in a substantial collection of melanoma lines expressing oncogenic BRAF, indicating that this is an inherent phenotype and may provide a biomarker for enhanced invasiveness and poor prognostic outcome. Indeed, in this regard, primary tumors showed higher overall PDE5A expression than did metastatic tumors. PDE5 downregulation was also clearly specific to BRAF mutant melanoma cells, as it was not evident in either NRAS mutant melanoma cells or BRAF mutant colorectal cells. The absence of PKG isoforms (PRKG1 and PRKG2) in melanoma cells may explain why loss of PDE5 does not confer relaxation, as the cGMP-activated kinase, PRKG1, mediates relaxation by cGMP in vascular smooth muscle cells (Weber et al., 2007).

Interestingly, Marais and colleagues found that in a melanoma cell line where PDE5A was not so strongly downregulated, inhibition of either MEK or Val600Glu-BRAF led to PDE5A upregulation and decreased invasiveness. This prompted them to question whether inhibition of PDE5 might promote an invasive phenotype in Val600Glu-BRAF-expressing melanoma cells where PDE5A was not fully downregulated. Indeed, this proved to be the case, with PDE5 inhibition promoting both in vitro and in vivo increased invasiveness of a Val600Glu-BRAF-expressing melanoma cell line where, although downregulated, significant PDE5A was still expressed. However, PDE5 selective inhibitors did not promote invasiveness in Val600Glu-BRAF- expressing melanoma cells where PDE5 expression was ablated. These data imply that means to upregulate PDE5A selectively in Val600Glu-BRAF-expressing melanoma cells may have therapeutic potential in preventing metastases.

Many questions remain to be resolved: one is the mechanism through which one of the ten Val600Glu-BRAF-containing melanoma cell lines investigated still showed significant PDE5 expression. Additionally, analyses of an extensive number of Val600Glu-BRAF-containing melanomas are needed to determine whether the particular melanoma cell line identified in these studies reflects an "oddity" where, normally, all oncogenic B-RAF melanomas are indeed PDE5A null and thus insensitive to any potential invasiveness-promoting effect that might be conferred through PDE5 inhibition. Investigations are also required to determine whether in Val600Glu-BRAF-expressing melanoma cells having residual PDE5A, chronic PDE5 inhibition is required to potentiate their invasiveness or whether acute PDE5 inhibition can suffice. It will also be important to determine whether such cells express other cGMP-hydrolyzing PDEs and, if so, whether their inactivation also promotes invasiveness or whether this phenotype is solely determined by a "pool" of cGMP that is specifically controlled by PDE5A. Certainly, such compartmentalization coupled to distinct phenotypic responses has been well characterized for cAMP pools determined by distinct PDE forms (Houslay, 2010). Finally, it will be of interest to determine whether PDE5 inhibition increases invasiveness of melanoma cells that do not express the mutant Val600Glu-BRAF or whether such a specific background is required for this action. Clearly these are important issues that require further investigation.

This elegant study thus provides an important insight into the mechanism whereby oncogenic B-RAF promotes invasiveness in melanoma cells through a mechanism that pivotally involves PDE5A downregulation and elevation of cGMP levels (Figure 1).

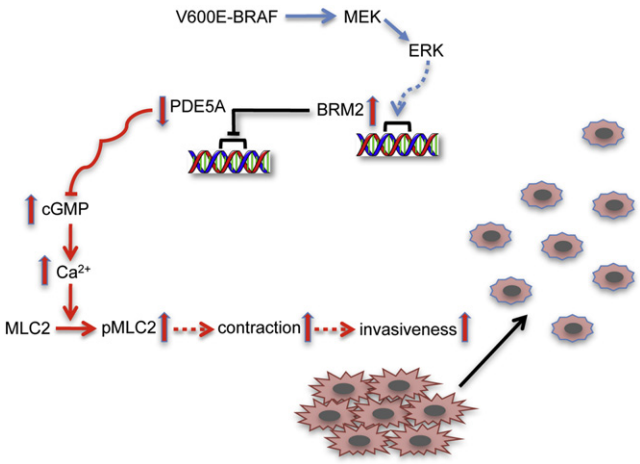


**Figure 1. PDE5A Downregulation in Val600Glu-BRAF-Expressing Melanoma Cells Promotes Invasiveness**

Schematic showing that expression of oncogenic Val600Glu-BRAF activates the ERK-MAPK pathways leading to upregulation of the BRN-2 transcription factor, which suppresses transcription of the *PDE5A* gene. Downregulation of PDE5A lowers cGMP degradation, causing an increase in intracellular cGMP levels. These elicit an increase in intracellular $Ca^{2+}$, which triggers the phosphorylation of myosin light chain 2, causing a contraction-induced shape change and increase in invasiveness.

## REFERENCES

Abadi, A.H., Gary, B.D., Tinsley, H.N., Piazza, G.A., and Abdel-Halim, M. (2010). Eur. J. Med. Chem. *45*, 1278–1286.

Arozarena, I., Sanchez-Laorden, B., Packer, L.M., Hidalgo-Carcedo, C., Hayward, R., Viros, A., Sahai, E., and Marais, R. (2011). Cancer Cell *19*, this issue, 45–57.

Bollag, G., Hirth, P., Tsai, J., Zhang, J., Ibrahim, P.N., Cho, H., Spevak, W., Zhang, C., Zhang, Y., Habets, G., et al. (2010). Nature *467*, 596–599.

Conti, M., and Beavo, J. (2007). Annu. Rev. Biochem. *76*, 481–511.

Francis, S.H., Corbin, J.D., and Bischoff, E. (2009). Handb. Exp. Pharmacol. *191*, 367–408.

Houslay, M.D. (2010). Trends Biochem. Sci. *35*, 91–100.

Kass, D.A., Champion, H.C., and Beavo, J.A. (2007). Circ. Res. *101*, 1084–1095.

Packer, L.M., East, P., Reis-Filho, J.S., and Marais, R. (2009). Pigment Cell Melanoma Res. *22*, 785–798.

Weber, S., Bernhard, D., Lukowski, R., Weinmeister, P., Worner, R., Wegener, J.W., Valtcheva, N., Feil, S., Schlossmann, J., Hofmann, F., and Feil, R. (2007). Circ. Res. *101*, 1096–1103.

Zhu, B., Vemavarapu, L., Thompson, W.J., and Strada, S.J. (2005). J. Cell. Biochem. *94*, 336–350.