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Melanoma, Viagra and PDE5 inhibitors: proliferation and metastasis

By

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Abstract

Inhibitors of PDE5 cGMP degrading phosphodiesterases have realised blockbuster status for treating penile erectile dysfunction. Indeed, their re-purposing is currently being proposed to treat certain solid tumours and various other diseases. In a cruel irony, however, it appears from recent clinical studies that PDE5 inhibitors may actually increase malignant melanoma risk by negating newly identified brakes on proliferation and metastasis provided by PDE5A.

Article Body

Things were simpler when cAMP and cGMP were the only second messenger signalling systems known. Now we are faced with a myriad of signalling systems such that trying to comprehend their integrated functioning is challenging both technically and intellectually. This is further complicated as their functioning is invariably tailored in a cell-type specific fashion to confer distinct modes of compartmentalization and regulation [1, 2].

Over the past few decades there has been an overwhelming focus of academic research on the more recently discovered 'trendy' signalling pathways, while the cAMP and cGMP pathways have had a more niche profile. In contrast, the pharmaceutical industry has been profitably mining the cyclic nucleotide area, generating a variety of blockbuster therapeutics. However, elucidation of the roles of cAMP and cGMP in the key areas of cell growth and proliferation has so far proved elusive, not least because they can elicit very different, and even diametrically opposed, actions in different cell types. There is a growing realisation of the importance of the need to understand this in order to aid in treating and diagnosing certain cancers. This will require analysis of cells in health and in disease, where effective therapy will require a personalized medicine approach.

It's becoming increasingly apparent that the cell type-specific behaviour of both cAMP and cGMP signalling is critically shaped by action of a super-family of cyclic nucleotide degrading phosphodiesterases (PDEs) differing in substrate specificity, mode of regulation and intracellular localization [1]. Of these, the cGMP specific PDE5A has attracted much attention because specific inhibitors, Viagra® (sildenafil), Zydena® (udenafil), Cialis® (tadalafil), and Levitra® (vardenafil), are effective in treating penile erectile dysfunction (PED). However, a few years ago it was suggested

that the therapeutic use of PDE5 selective inhibitors may negatively impact on malignant melanoma, an extremely aggressive cancer [3]. Indeed, this prediction appears to have credence as two recent clinical studies indicate that PDE5 inhibitor usage causes a modest increase in the risk of melanoma [4, 5].

What links PDE5 to melanoma? The first mechanistic insight came from observations [3] that PDE5A was down-regulated in a large number of melanoma lines expressing oncogenic Val600Glu-BRAF where cells adopt a de-differentiated, metastatic phenotype. The underpinning mechanism involved Val600Glu-BRAF driven Erk activation causing up-regulation of BRN-2, which inhibits PDE5A transcription and so attenuates cGMP degradation. Increased cGMP, caused by PDE5A loss, elicits phenotypic change via Ca^{2+} elevation and MLCK phosphorylation. Here, PDE5 inhibitors have little impact on the majority of Val600Glu-BRAF-melanomas because PDE5 has undergone severe down-regulation, but does impact to promote metastasis on the small population where significant PDE5 remains.

The second key insight has just been uncovered [6]. This involves a novel cGMP signalling pathway in melanoma cells lacking an activating BRAF mutation. Here, a large increase in cGMP is elicited through activation of membrane-bound guanylyl cyclase B (GCB) by pro-melanomagenic C-type natriuretic peptide (CNP), which is released by the tumour vasculature, particularly under inflammatory conditions. Through an unknown means, activated cGMP kinase-Iα (PKG-Iα) stimulates Erk signalling, promoting melanocyte proliferation rather than promoting a metastatic phenotype. In these melanomas, inhibiting cGMP degradation through PDE5 inhibition enhances the growth promoting effect of CNP and thus tumour size.

In both these instances PDE5 provides a brake that, depending on the melanoma type, constrains either proliferation or de-differentiation to a metastatic phenotype. PDE5 inhibitors can release this brake.

It may be that cGMP elevation in a sub-compartment controlled by PDE5A leads to a metastatic signal, while cGMP elevation due to GCB activation in a distinct sub-compartment leads to proliferative signal that is merely amplified upon PDE5A inhibition. However, other complicating factors probably come into play here. Interestingly, melanomas contain phenotypically mixed melanocyte populations where hypoxic microenvironments provide an important driving factor in this heterogeneity, influencing proliferation and metastasis [7]. Indeed, hypoxia potently reduces expression of microphthalmia-associated transcription factor (MITF), the master gene of melanoma differentiation, through the Hif1α induction of the transcriptional repressor, DEC1 [8]. Melanocytes with low MITF levels exhibit a de-differentiated, invasive

phenotype, while those with high MITF do not [7]. Intriguingly, it is possible to relate this back to these recent key discoveries concerning PDE5A. This is because high MITF increases levels of the pigment-cell-enriched microRNA, miR-211, which originates from the TRPM1 (melastatin) gene. This represses BRN-2 [9], the transcriptional repressor of PDE5A [3]. Thus hypoxic melanoma microenvironments generate sub-populations of melanocytes with low MITF and PDE5A levels. The ensuing elevated cGMP in the PDE5A sub-compartment consequently promotes the metastatic phenotype. Conversely, cells with high MITF will be protected against de-differentiation, retaining both a proliferative phenotype and sensitivity to the growth promoting action of PDE5 inhibitors.

Clearly it is critical to appreciate the role of PDE5A and action of all therapeutic PDE5 inhibitors in melanocytes expressing a range of oncogenic mutations. Associated with this, there is a need to identify the PKG isoforms expressed in such cells, their compartmentalisation and relationship to PDE5A, as recent evidence indicates that while PKG-Iα activation promotes cell growth PKG-Iβ inhibits it [6]. As PDE5 is not the only cGMP degrading PDE, it is also important to determine which other such PDEs melanocytes express and if their inhibition exerts effects similar to those of PDE5 inhibitors or whether they control functionally distinct pools of cGMP.

As all PDE5 selective inhibitors have potential to release a brake provided by PDE5A on deleterious proliferation and metastasis in certain melanomas, it will be important to consider having prospective clinical studies undertaken to assess the significance of this potential issue. These should have clearly defined inclusion and exclusion criteria and set doses of various PDE5 inhibitors in order to determine whether any recommendation should be made regarding their clinical use. PDE5 inhibitors are not used chronically in PED and are usually cleared rapidly. However, careful consideration of the 'melanoma question' will have to be given regarding the other therapeutic indications that PDE5 inhibitors are either used in now or are being considered as these will involve chronic administration and high systemic [10].

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Keywords.
Melanoma; cyclic GMP; PDE5 ; Sildenafil; Viagra; hypoxia.

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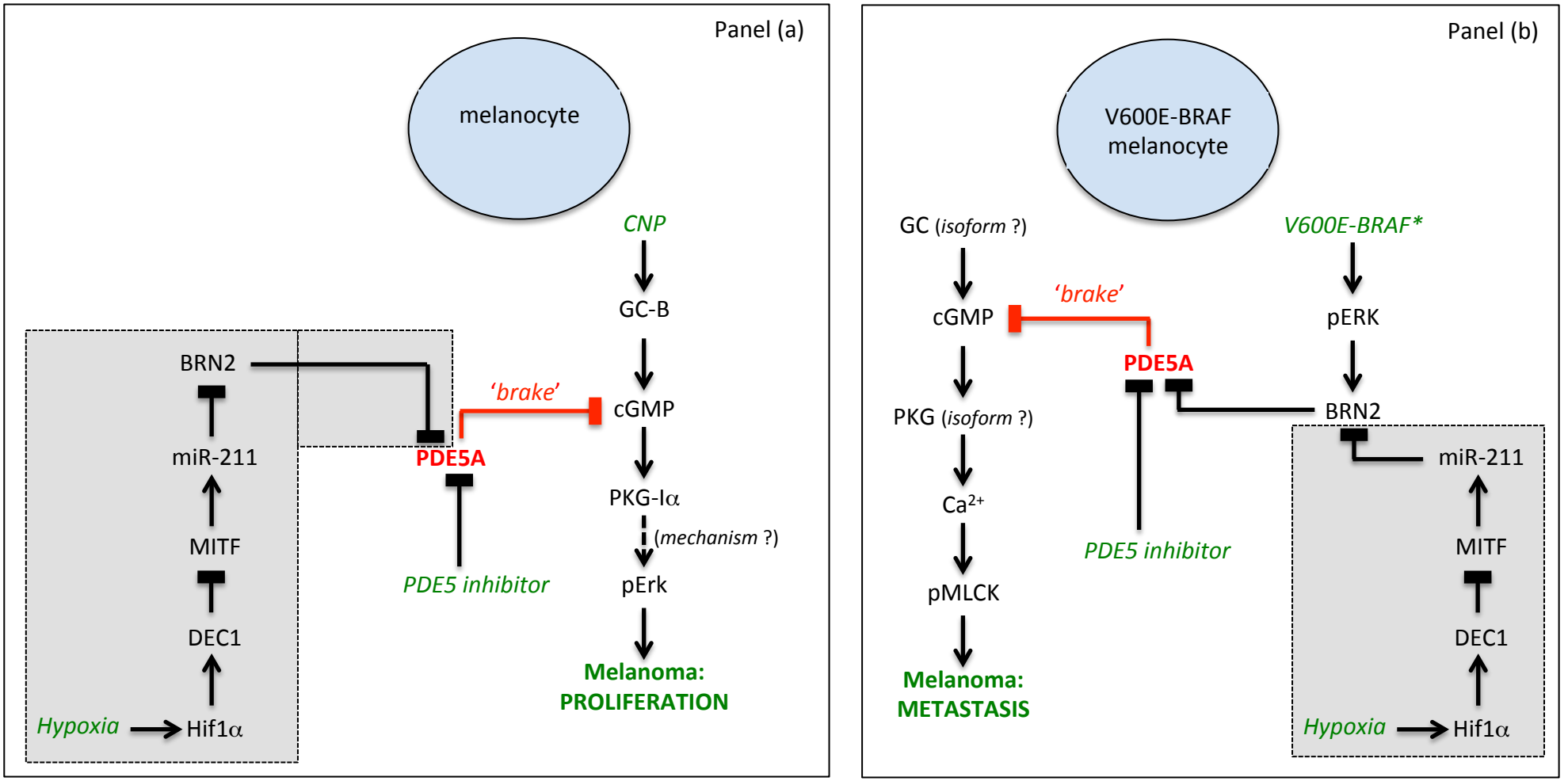
Figure.

PDE5 at the centre of Phenotype Switching in Melanoma.

Panel (a). In melanocytes lacking activated V600E-BRAF then CNP activated guanylate cyclase B increases cGMP, which elicits a proliferative (GREEN) response through an undefined PKG-Iα / Erk – driven pathway. A brake (RED) is imposed on this by PDE5, which can be overcome by PDE5 inhibitors. It is proposed here that hypoxia (GREY BOX) may also amplify switching to a proliferative phenotype, in this case by lowering MITF levels in a Hif1a-driven fashion in order to remove the inhibitory effect of miR-211 on BRN2 transcription and so decreasing PDE5A

expression. (RED = brake supplied by PDE5; GREEN = proliferative phenotype switching signals provided by PDE5 inhibitors and, potentially, hypoxia).

Panel (b). In V600E-BRAF expressing melanocytes, the brake (red) that PDE5A exerts on cGMP driven metastasis is released when PDE5 levels are diminished through activation of the transcriptional repressor, BRN2 via an activated BRAF/Erk pathway. Populations of V600E-BRAF expressing melanocytes show variation in the level of PDE5 reduction and those expressing residual PDE5 will be susceptible to the action of PDE5 inhibitors, which will potentiate the development of a de-differentiated, metastatic phenotype. It is proposed here that hypoxia (GREY BOX) may also amplify switching to a metastatic phenotype, in this case by lowering MITF levels in a Hif1a-driven fashion in order to remove the inhibitory effect of miR-211 on BRN2 transcription and so decreasing PDE5A expression. (RED = brake supplied by PDE5; GREEN = metastatic phenotype switching signals provided by PDE5 inhibitors, activated V600E-BRAF and, potentially, hypoxia).


Panel (a)
melanocyte
CNP
GC-B
cGMP
PKG-Iα
(mechanism ?)
pErk
Melanoma:
PROLIFERATION
'brake'
PDE5A
PDE5 inhibitor
BRN2
miR-211
MITF
DEC1
Hypoxia
Hif1α
Panel (b)
V600E-BRAF
melanocyte
GC (isoform ?)
cGMP
PKG (isoform ?)
Ca2+
pMLCK
Melanoma:
METASTASIS
'brake'
PDE5A
PDE5 inhibitor
V600E-BRAF*
pERK
BRN2
miR-211
MITF
DEC1
Hypoxia
Hif1α