PTEN as a target in melanoma

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ABSTRACT

PTEN is a well-known tumour suppressor protein that is frequently found to be mutated, inactivated or deleted in a wide range of different cancers. Its tumour suppressive properties result predominantly from its inhibitory effects on the PI3K-AKT signalling pathway. In melanoma, numerous different PTEN mutations have been identified in both melanoma cell lines and melanoma tissue. A number of different molecules can act on PTEN to either promote its suppression of melanoma, while other molecules may antagonise PTEN to inhibit its mechanism of action against melanoma. This review will discuss how the interactions of PTEN with other molecules may have a positive or negative impact on melanoma pathogenesis, giving rise to the potential for PTEN-targeted therapies against melanoma.

BACKGROUND

Melanoma is the most fatal form of skin cancer, and mortality rates continue to increase. In the case of Australia, melanoma is the fourth most commonly diagnosed cancer overall and the most common cancer in young adults.[1] In the absence of complete excision or successful treatment, melanoma cells will continue to proliferate. This allows for penetration through to the dermis of the skin, providing physical access to the blood and lymphatic vessels through which melanoma cells can metastasise to other organs such as the liver, lung, brain and bones to form secondary tumours.[2]

PI3K-AKT PATHWAY AND PTEN

AKT, also known as protein kinase B, is an oncoprotein involved in regulation of cellular proliferation, survival, motility and angiogenesis.[3] AKT signalling is overactivated in many cancer types, including melanoma,[4] glioblastoma,[5] prostate cancer[6] and thyroid cancer.[7] This abnormal regulation of the AKT pathway favours survival and proliferation of cancer cells. AKT is a key player in the phosphoinositide 3-kinase (PI3K)-AKT signalling pathway. As summarised in figure 1, this pathway is initiated by the binding of growth factors, for example, epidermal growth factor, to receptors on the surface of the cell, leading to the conversion of RAS from its guanosine diphosphate (GDP) bound form to guanosine triphosphate (GTP) bound form. RAS can then activate the catalytic activity of PI3K. Activated PI3K phosphorylates phosphatidylinositol 4,5-bisphosphate (PIP2) at the plasma membrane to phosphatidylinositol (3,4,5)-triphosphate (PIP3). This then recruits AKT to phosphoinositide dependent kinase 1 (PDK1) and mechanistic target of rapamycin complex 2 (mTORC2) to activate AKT via phosphorylation at two residues, Thr308 and Ser473.[8 9] Activated AKT can act on its many substrates involved in the regulation of survival (BAD, caspase 9), growth (mTOR), motility and angiogenesis.[10 11] The tumour suppressor, phosphatase and tensin homolog deleted on chromosome 10 (PTEN), is largely responsible for the negative regulation of the PI3K-AKT pathway through dephosphorylation of PIP3 to PIP2 in the cytoplasm (figure 1).[12]

Excessive exposure to ultraviolet radiation from the sun can cause high levels of irreparable DNA damage which can lead to the accumulation of mutations in key tumour suppressor genes, including PTEN, as benign nevi progress to melanoma.[13 14] In fact, increases in activity of the PI3K-AKT pathway are mainly due to mutations in PTEN.[15] PTEN mutations have been observed in a number of cancers such as bone,[12] breast,[16] thyroid[17] and melanoma.[18] These mutations can lead to the inactivation or deletion of functional PTEN.[18] Indeed, loss of PTEN function has been reported in 35% of melanomas and is often found in late stage melanomas alongside BRAF mutations.[19] As a result, the PTEN regulation of PIP3 is minimised, leading to abnormally high levels of activated AKT which can give rise to a favourable tumour microenvironment.[20] Clinical studies have implicated the RAS-RAF-MEK-ERK signalling pathway in the development of melanoma, with mutations in BRAF and NRAS commonly found in melanoma patients.[21 22] However, a number of studies suggest that a mutation in this pathway alone is insufficient for melanoma progression, since BRAF and NRAS mutations are also common in benign nevi.[23] Indeed, mutations in the PI3K-AKT pathway and downstream targets have been linked with melanoma progression.[24–26]

C-JUN PROMOTES THE SURVIVAL OF MELANOMA CELLS VIA THE PI3K-AKT PATHWAY

It is known that c-Jun promotes cancer cell survival through the negative regulation of PTEN.[27] Studies performed by Ciuffreda *et al* demonstrated this relationship in the human M14 melanoma cell model.[28] Treatment with the MEK/ERK pathway inhibitor PD0325901 caused an upregulation of PTEN in these cells and a downregulation of c-Jun in a time-dependent and dose-dependent manner. This result was also observed in other melanoma cell lines as well as breast cancer and myeloid leukaemia cell lines, accompanied by an inhibition of AKT phosphorylation. They confirmed that the effects of PD0325901 on PTEN and c-Jun levels were indeed through the inhibition of the MEK/ERK pathway. Collectively, these findings strongly suggest that the MEK/ERK pathway in melanoma is involved in PTEN inhibition via c-Jun activation, which in turn activates the PI3K/AKT pathway, leading to melanoma cell survival and likelihood of progression.[28]

A more recent study by Kappelmann-Fenzl *et al* showed that PTEN may be targeted during early

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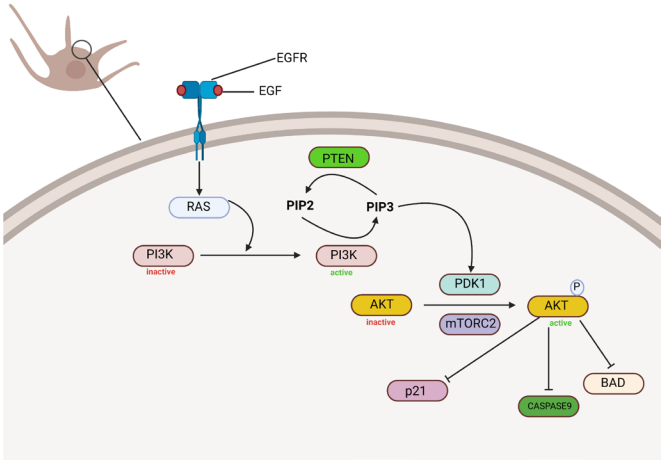

Figure 1 PI3-AKT signalling pathway. Growth factors, for example, epidermal growth factor (EGF) bind to receptors on the cell. RAS is converted from its GDP to GTP bound form. RAS activates the catalytic activity of PI3K. PI3K converts PIP2 into PIP3 at the plasma membrane, leading to recruitment of PDK1 and AKT to the membrane. PDK1 and mTORC2 activate AKT via phosphorylation. PTEN can inhibit AKT activity through desphosphorylation of PIP3 to PIP2. GDP, guanosine diphosphate; GTP, guanosine triphosphate; mTORC2, mechanistic target of rapamycin complex 2; PDK1, phosphoinositide dependent kinase 1; PI3K, phosphoinositide 3-kinase; PIP2, phosphatidylinositol 4,5-bisphosphate; PIP3, phosphatidylinositol (3,4,5)-triphosphate; PTEN, phosphatase and tensin homolog deleted on chromosome 10. Created with biorender.com.

melanoma development in order to suppress its tumour suppressive functions on the PI3K-AKT pathway.[29] They demonstrated a positive correlation between PTEN and c-Jun, a member of the AP-1 transcription factor family, in melanoma cell lines. Indeed, levels of c-Jun expression were highest in $PTEN^{WT}$ cells, with lower levels of c-Jun in $PTEN^{HemDel}$ and even lower levels again in $PTEN^{HomDel}$ cells, indicating a dependence of c-Jun upregulation on PTEN expression status.[29] This overpowering of PTEN by c-Jun was evidenced by similar levels of AKT detected in $PTEN^{WT}$ cells and $PTEN^{HomDel}$ cells. Additionally, they showed via RNA-seq that several of the PI3K-AKT target genes expressed in $PTEN^{HomDel}$ melanoma cells were also found to be expressed in $PTEN^{WT}$ melanoma cells. Their studies suggest that c-Jun can overpower the tumour suppressive function of PTEN to favour survival pathways in the early stages of melanoma development, and raise the possibility of targeting c-Jun in $PTEN^{WT}$ melanoma cells to inhibit melanoma cell survival.[29]

MIR-367 PROMOTES THE PROGRESSION OF MELANOMA THROUGH THE REGULATION OF PTEN

Recent studies have linked PTEN and miRNA-367 (miR-367) in melanoma. MicroRNAs (miRNAs) are small, non-coding RNA molecules that play an important role in regulating gene expression.[30] One such molecule, miR-367 has been observed in the progression of different cancers[31–33] and has been shown to be overexpressed in melanoma.[33] In the study by Long *et al*, miR-367 levels were found to be significantly higher in human cutaneous melanoma tissue when compared with benign nevi tissue. Similarly, human melanoma cell lines had significantly higher levels of miR-367 when compared with human melanocytes.[33] The elevated levels of miR-367 in melanoma tissue was accompanied by low levels of PTEN and associated with poor prognosis. They confirmed PTEN as a downstream target of miR-367, showing that miR-367 could directly bind to PTEN and regulate its activity. Indeed, forced expression of miR-367 in a melanoma cell line decreased PTEN expression, leading to activation of AKT.[33]

In a separate study, PTEN was also implicated as a direct target of miR-367 in uveal melanoma. It was shown that miR-367 expression was significantly greater in uveal melanoma cell lines and uveal melanoma samples in comparison to normal uveal melanocytes and normal uveal specimens, respectively.[34] Transfection of the M23 uveal melanoma cell line, which had relatively low baseline miR-367 levels when compared with the other uveal melanoma cells, with a miR-367 mimic, led to reduced PTEN levels and increased proliferation and migration properties. However, when this mimic was cotransfected with PTEN lacking the miR-367 binding sequence, proliferation and migration of the M23 cells was markedly decreased.[34]

Long intergenic noncoding RNA 00961 (Linc00961) is a known tumour suppressor in multiple cancers.[35 36] It has been shown to be downregulated in melanoma tissue from patients and in melanoma cell lines, compared with tissue from benign nevi and normal melanocytes respectively.[36] In the same study, overexpression of Linc00961 in a number of melanoma cell lines inhibited cellular proliferation, decreased migration and invasion, and promoted apoptosis.[36] Interestingly, overexpression of Linc00961 in melanoma cell lines was found to significantly downregulate the expression of miR-367, through Linc00961 sponging miR-367. It was further shown that Linc00961 could upregulate expression of PTEN, a known target of miR-367. Moreover, silencing of PTEN in cells overexpressing Linc00961 restored the inhibitory effects on proliferation and migration, illustrating the importance of PTEN in the tumour suppressive action of Linc00961 in melanoma.[36]

MELANOCORTIN-1 RECEPTOR POSITIVELY REGULATES THE ACTIVITY OF PTEN IN MELANOCYTES

Melanocortin-1 receptor (MC1R) is a G protein-coupled receptor mainly found in melanocytes and has a role in the process of pigmentation.[37 38] MC1R activation is dependent on binding to its ligand, α-melanocyte stimulating hormone (α-MSH), which occurs on the exposure to ultraviolet B (UVB) radiation[39] (figure 2). Mutations of MC1R, when compared with wild-type MC1R, result in less effective cyclic AMP (cAMP) production following α-MSH binding. As a result, pigmentation is decreased.[37]

Studies by Cao *et al* showed that wild type MC1R (WT-MC1R) is a positive regulator of PTEN activity in melanocytes following exposure to UVB[37] (figure 2). Using K14/SCF transgenic mice containing either WT-MC1R or a loss-of-function MC1R mutation, it was found that following exposure to UVB, there was a significant increase in the inactivating phosphorylation of PTEN (Ser380 and Thr382/383) and activating phosphorylation of AKT (Ser473) in the mouse skin containing mutated MC1R. Similarly, in *in vitro* studies, MC1R-depleted melanocytes had elevated levels of inactivated PTEN and activated AKT.[37] Furthermore, UVB exposure triggered MC1R-PTEN interaction in WT-MC1R melanoma cells, but not in cells containing MC1R with RHC traits (red hair colour, fair skin, poor tanning). The positive interaction between MC1R and PTEN suggests a protective role for MC1R in UVB-induced PTEN degradation, thereby preventing uncontrolled activation of AKT that would favour melanoma development. Their observations suggest that

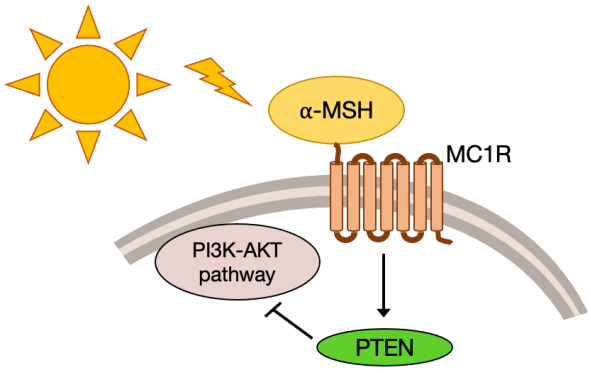

Figure 2 WT-MC1R activation of PTEN. On ultraviolet B (UVB) radiation exposure, α-melanocyte stimulating hormone (α-MSH) expression is markedly increased. MCR1 is activated on binding to α-MSH and can stimulate pigmentation. WT-MC1R activation also induces PTEN activity which suppresses the PI3KAKT pathway through its phosphatase activity on PIP3. MC1R, Melanocortin-1 receptor; PI3K, phosphoinositide 3-kinase; PIP3, phosphatidylinositol (3,4,5)-triphosphate; PTEN, phosphatase and tensin homolog deleted on chromosome 10; WT, wild type.

this indeed may be the case as it was found that UVB-induced PTEN ubiquitination was significantly higher in melanocytes with depleted levels of MC1R when compared with melanocytes treated with control small hairpin RNA.

The findings above are supported by studies using synthetic analogue of α-MSH, Melanotan II (MTII), in a B16-F10 melanoma model.[40] Treatment of B16-F10 cells using MTII significantly decreased levels of cellular migration and invasiveness. Similarly, in a B16-F10 murine model of primary melanoma, topical MTII treatment significantly reduced tumour sizes compared with control mice. As aforementioned, α-MSH-induced MC1R activation gives rise to elevated levels of activated PTEN. Indeed, MTII treatment of the B16-F10 melanoma cells caused significant increases in the levels of active PTEN with concomitant decreases in inactivated PTEN in a dose-dependent manner. These results raise the possibility of targeting PTEN using agents such as MTII to attenuate the PI3K-AKT pathway and hence reduce the aggressiveness of melanoma.

CONCLUSIONS AND SIGNIFICANCE

This review highlights the critical role of PTEN, and its effects on PI3K-AKT pathway regulation, in melanoma development. Interaction of PTEN with its antagonists such as c-Jun and miR-367 can decrease PTEN activity, leading to increased AKT phosphorylation and subsequent effects on promotion of cellular survival, proliferation and migration of melanoma cells. However, by increasing activation of PTEN, for example by MC1R activation in melanoma, cell survival pathways are compromised. The studies reviewed here raise the possibility of targeting PTEN in melanoma. Such targeted therapies could directly act on PTEN to promote its activity or indirectly act on PTEN via positive or negative interactions with its synergistic or antagonistic partners, respectively. Indeed recent studies demonstrate that the active metabolite of vitamin D, 1,25-dihydroxyvitamin D3 can reduce melanoma cell viability in a PTEN-dependent manner.[41]

Although promising, studies on the interactions of PTEN with other molecules in the context of melanoma are not highly extensive and as such, further studies are required to increase the plausibility of PTEN-targeted melanoma therapy. This may indeed uncover other PTEN associations and downstream targets, which could in turn lead to improved and more efficient therapeutic strategies for melanoma.

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