Sphingosine kinase 1 enables communication between melanoma cells and fibroblasts that provides a new link to metastasis

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ABSTRACT--In this issue of Oncogene, Albinet and colleagues have demonstrated a critical role of melanoma sphingosine kinase 1, which catalyses formation of sphingosine 1-phosphate, in promoting the differentiation of fibroblasts into myofibroblasts. The myofibroblast sphingosine kinase 1 then promotes the sphingosine 1-phosphate-dependent dissemination (metastasis) of melanoma cells via a sphingosine 1-phosphate receptor 3-mediated mechanism. These findings are of major significance because they provide a novel mechanism of interaction between melanoma and the microenvironment niche in promoting metastasis. These studies therefore identify sphingosine 1-phosphate derived from myofibroblasts and melanoma cells as a novel target for therapeutic intervention.

Sphingosine 1-phosphate (S1P) is a bioactive lipid that has been demonstrated to have an important role in cancer including the promotion of metastasis of tumour cells[1]. Recently Ponnusamy et al.[2] demonstrated that systemic S1P, produced by the host sphingosine kinase 1 (SPHK1) rather than tumour-derived S1P, promotes lung colonisation/metastasis. Thus, a reduction in systemic S1P inhibited TRAMP-induced prostate cancer growth in *TRAMP*$^{+/+}$*Sphk1*$^{-/-}$ mice or lung metastasis of a number of cancer cells in *Sphk1*$^{-/-}$ mice. The loss of SPHK1 promotes the expression of *Brms1* (breast carcinoma metastasis suppressor 1). Indeed, S1P binding to S1P receptor 2 ($S1P_2$) reduces *Brms1* expression in cancer cells. The sequestration of systemic S1P using a novel anti-S1P monoclonal antibody also reduced lung metastasis and this was prevented by knockdown of *Brms1* in the tumour cells[2]. These findings raise the question of the source of systemic S1P. The major advance made by Albinet et al.[3] in this issue of Oncogene is that the S1P derived from myofibroblasts in the tumour microenvironment might be a potential source of systemic S1P. It has been well established that stromal cells functionally interact with tumour cells to promote aggressive metastatic cancer. The stroma is principally composed of myofibroblasts (also known at tumour associated fibroblasts, TAF) which are derived from fibroblasts, homing fibrocytes and/or mesenchymal cells. The TAFs secrete growth factors and

extracellular matrix and matrix metalloproteinases, which promote cancer progression. Indeed, the dense fibrotic nature of melanoma is thought to be a consequence of myofibroblasts and poor prognosis is linked with the presence of these cells in the tumour.

This then raises the question as to the role of the myofibroblasts within the context of S1P biology and melanoma dissemination. In this regard, there is a substantial body of evidence demonstrating that S1P is involved in fibrosis; promoting differentiation of fibroblasts into myofibroblasts. For instance, S1P increases the expression of SPHK1 and $S1P_{1/3}$ which are increased in human fibrotic livers[4]. In addition, siRNA knockdown of SPHK1 reduces TGFβ1-stimulated α-SMA and fibronectin expression in WI-38 human lung fibroblasts; a differentiation response that is mediated by S1P and $S1P_{2/3}$ receptors[5]. TGFβ also increases SK1 expression in cardiac fibroblasts[6]. In this case, S1P formed by SPHK1 is released from cardiac fibroblasts and stimulates collagen and α-SMA expression via an $S1P_2$- and Rho kinase-dependent pathway in an autocrine manner[6].

Albinet et al.[3] have connected SPHK1 expressed in melanoma with SPHK1 in myofibroblasts to provide a molecular explanation for the dissemination and growth of melanoma (Fig. 1). These authors demonstrate that S1P metabolism is disrupted in melanoma cells. For instance, the expression level of SPHK1 is substantially increased in melanoma compared with adjacent melanocytic nevus in patients. Moreover, over-expression of WT BRAF or BRAFV600E constitutively active mutant in HEK 293 cells induced an increase in the expression of SPHK1 and this is dependent on ERK activation. In addition, siRNA knockdown of BRAF in SKMel-28 cells reduced ERK phosphorylation and SPHK1 expression. These data are important as they link oncogenic BRAF, a major driver of melanoma with changes in SPHK1 expression. The authors then used COLO829 melanoma cells, which are SPHK1 null to generate ectopic SPHK1

expressing COLO829 cells, and found that the migration of these cells was unaffected. The lack of a role for SPHK1 in regulating melanoma migration was confirmed using SPHK1 silencing in A375 melanoma cells. These surprising findings strongly support a model in which S1P formed by melanoma cells, where SPHK1 expression is increased, does not function in an autocrine manner to stimulate cell migration via S1P receptors. In contrast, exogenous S1P did promote the migration of A375 cells via an $S1P_{1/3}$-dependent mechanism. However, using conditioned medium from SPHK1 expressing COLO829 cells (compared with COLO829 cells, SPHK1 null), Albinet et al.[3] demonstrated the differentiation of skin fibroblasts into myofibroblasts with increased expression and release of MMP-2 and MMP-9, both of which are involved in melanoma cell invasion. Conditioned medium from SPHK1 expressing COLO829 cells also increased the expression of SPHK1 in fibroblasts and this resulted in increased S1P release from these fibroblasts. These findings were recapitulated in TAF compared with human dermal fibroblasts. Therefore, S1P released from melanoma cells appears to promote a fibrotic response in the tumour microenvironment. Significantly, conditioned medium from SPHK1 over-expressing fibroblasts promote A375 melanoma migration, while a 50% knockdown of SPHK1 in fibroblasts decreased melanoma migration. The conditioned medium from SPHK1 over-expressing fibroblasts also induced a 3-fold increase in $S1P_3$ expression in melanoma cells, suggesting that the migratory response of these cells is mediated by this receptor. These findings are in line with the observation that mice allografted with B16F10 melanoma exhibited substantial retardation of tumour progression upon anti-S1P antibody treatment[7].

Finally, Albinet et al.[3] injected B16F10 murine melanoma cells into the dermis of wild-type or *Sphk1*$^{-/-}$ mice together with WT or *Sphk1*$^{-/-}$ dermal fibroblasts. Melanoma growth was significantly reduced in *Sphk1*$^{-/-}$ syngenic mice. Moreover, tumour volume and weight was reduced when B16F10 murine melanoma cells were injected with *Sphk1*$^{-/-}$ dermal fibroblasts

compared with WT fibroblasts. 15-20% of WT mice inoculated with B16F10 murine melanoma cells with WT fibroblasts developed lung metastasis, while 2.5% of the mice developed metastasis when *Sphk1*$^{-/-}$ fibroblasts were used. The number of lung metastases was four times higher in mice implanted with B16F10 murine melanoma cells with WT fibroblasts compared with animals coinjected with *Sphk1*$^{-/-}$ fibroblasts. Therefore, melanoma invasiveness is critically dependent upon S1P derived from fibroblast over-expressing SPHK1. Consistent with these observations, $S1P_1$ signalling in myeloid cells activates fibroblasts in distant organs and promotes the formation of a pre-metastatic niche[8]. S1P lyase (SGPL1), the enzyme that catalyses the degradation of S1P, is also down-regulated in metastatic and invasive melanoma cells and this is likely to enhance S1P accumulation as a consequence of the BRAF-dependent up-regulation of SPHK1 in these cells. Moreover, SGPL1 silencing in fibroblasts increases melanoma cell migration[3].

There are many thought provoking questions raised by the elegant study of Albinet et al.[3]. First, it is unclear why S1P formed from SPHK1 in the melanoma cells does not act in an autocrine manner to stimulate melanoma migration. The authors plausibly argue that $S1P_3$ expression levels are not sufficiently high to promote migration, and that S1P released from the myofibroblast induces up-regulation of $S1P_3$ expression in the melanoma cells to confer sensitivity to migration. However, it is possible that S1P released from melanoma cells is not accessible to melanoma $S1P_3$ receptors, especially if the S1P transporters are positioned close to a melanoma-fibroblast contact, where S1P has immediate access to fibroblast S1P receptors. It is therefore important to determine whether $S1P_3$ is the principle receptor mediating differentiation of fibroblasts into myofibroblasts or whether SPHK1 promotes the release from melanoma cells of alternative fibrotic factors, such as TGFβ. This would also provide an explanation for the lack of a direct effect of melanoma SPHK1 on migration.

Interestingly, we have shown that S1P promotes translocation of SPHK1 to the plasma-membrane of MCF-7 breast cancer cells via an $S1P_3$-dependent mechanism[9]. In addition, siRNA knockdown of SPHK1 reduces $S1P_3$ expression in these cells and this results in reduced S1P/$S1P_3$-stimulated ERK-1/2 activation and abrogated migration of MCF-7 cells[9]. Therefore, SPHK1 regulates responsiveness of these cancer cells by fine-tuning the expression level of $S1P_3$. It is possible that a similar 'sensor' mechanism operates between TAF and melanoma cells regulated by TAF released S1P and melanoma SPHK1. This might produce a positive amplification loop of $S1P_3$-mediated invasive signalling in the melanoma cell. It would be interesting to establish the effect conditioned medium from differentiated fibroblasts on the migration of melanoma cells in which SPHK1 has been silenced.

Second, the studies of Albinet et al.[3] raise the question as to whether the communication between TAF and melanoma cells is therapeutically exploitable. Clearly, anti-S1P immunotherapy might sequester S1P released from both TAFs and melanoma cells to disrupt the communication, although this requires formal testing. Further consideration of the effect of depleting systemic S1P on immune surveillance is also required. $S1P_3$ antagonists might also be useful in preventing TAF-derived S1P driven melanoma invasiveness. Finally, SPHK1 is a major therapeutic target. We have shown that SPHK inhibitors, such as SKi (2-(p-hydroxyanilino)-4-(p-chlorophenyl)thiazole) and FTY720 induce the ubiquitin-proteasomal degradation of SK1 in androgen-sensitive prostate cancer and MCF-7 breast cancer cells. This results in an increase ceramide levels in androgen-sensitive prostate cancer cells which induces apoptosis[10]. Liang and colleagues have also demonstrated that FTY720 reduces colitis-induced cancer via down-regulation of SPHK1 expression that eliminates a malicious amplification loop involving SPHK1/$S1P_1$ and STAT3/IL-6 formation[11]. However, an N-terminal variant of SPHK1 (termed

SPHK1B) in androgen-independent prostate cancer cells evades the proteasome in response to SPHK1 inhibitors and this confers resistance to apoptosis[10]. In addition, a novel SPHK1 inhibitor, PF-543 with nM potency fails to induce apoptosis of certain cancer cells, although this compound does not increase ceramide levels indicating that it might modulate additional off-targets that neutralise the effect of SPHK1 inhibition[12]. Therefore, in certain cancer cells, the N-terminal variant of SPHK1 might confer resistance to SPHK1 inhibitors. The question then is whether SPHK1 in fibroblasts can also confer resistance to these inhibitors. If this is not the case, then such inhibitors could potentially ablate communication between fibroblasts and melanoma cells to prevent metastasis. Indeed, chemoresistance is generally more prominent in cancer cells and the link with fibroblasts provides a new opportunity to indirectly attack melanoma. Therefore, the ground breaking studies of Albinet et al.[3] provide substantial impetus to the development of anti-S1P therapeutics in cancer.

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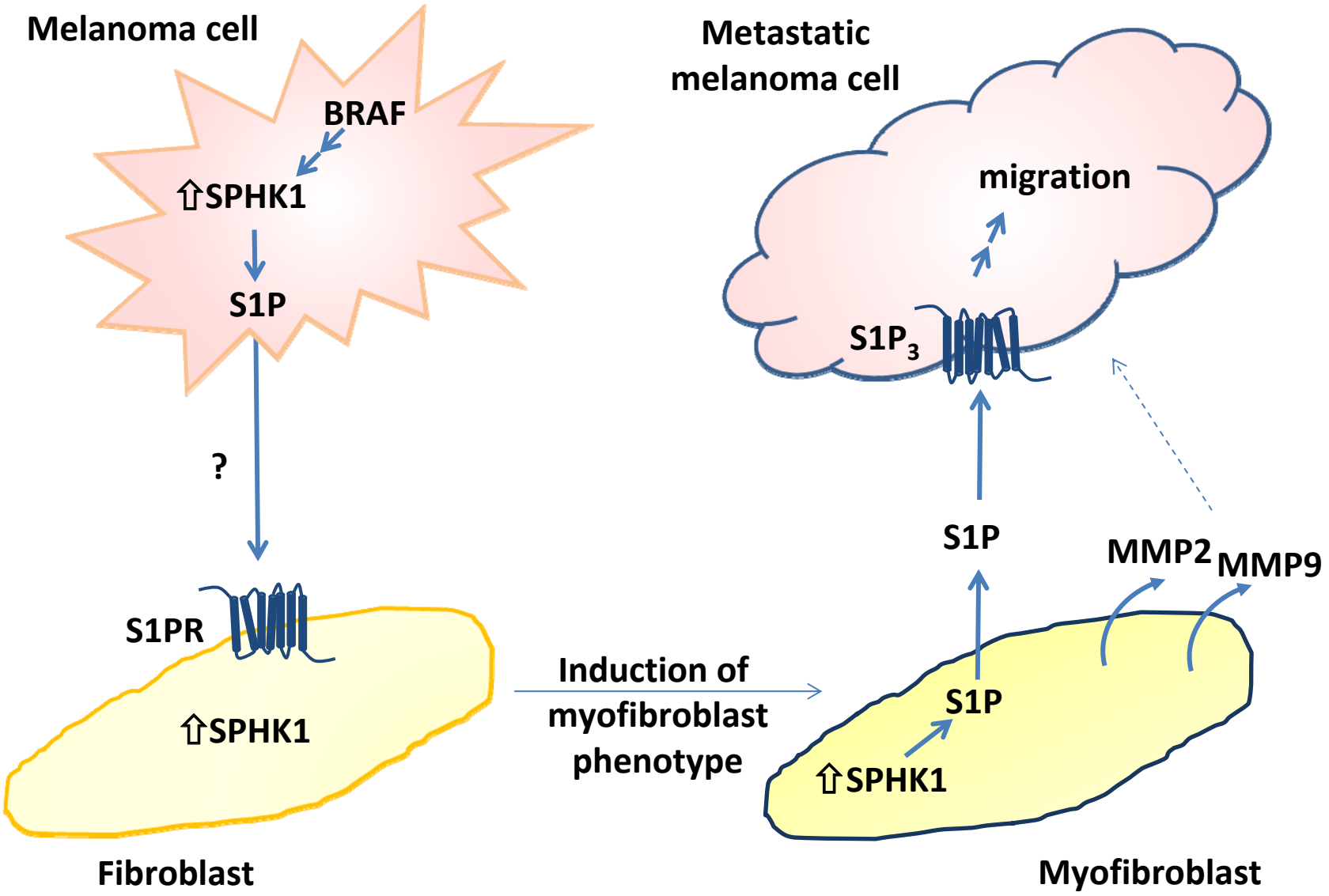
Fig. 1 Schematic showing the dual role of SPHK1 in regulating the interaction between melanoma cells and tumour associated fibroblasts which promotes metastasis. The melanoma cell releases S1P/fibrotic factors under the regulatory control of SPHK1 (expression of which is regulated by BRAF) and this promote fibroblast/myofibroblast transition. The SPHK1 in myofibroblasts then produces S1P which is released to bind to $S1P_3$ receptors on the melanoma cell to promote dissemination (metastasis) of the cancer. Therefore, SPHK1 performs a dual function and is a viable novel target for therapeutic intervention in melanoma.

Fig. 1


Melanoma cell
BRAF
⇧SPHK1
S1P
?
S1PR
⇧SPHK1
Fibroblast
Induction of
myofibroblast
phenotype
Metastatic
melanoma cell
migration
S1P3
S1P
MMP2
MMP9
S1P
⇧SPHK1
Myofibroblast