Melanoma's fatal attraction to lysophosphatidic acid trails: a new prognostic and therapeutic approach?

“We speculate that some aspects of the lysophosphatidic acid axis may provide novel prognostic and therapeutic markers.”

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Early-stage cutaneous melanoma is curable but advanced, metastatic melanoma is almost uniformly fatal [1]. Patients with such advanced disease have a short median survival of only 6–9 months [2]. Not only is cutaneous melanoma responsible for 75–80% of skin cancer deaths but there is an ongoing ‘melanoma epidemic’ [3].

Detecting and excising melanoma early is key to improving survival and melanoma is characterized by clinical and biological heterogeneity, which in part explains why melanoma is notoriously resistant to chemo- and immuno-therapeutic strategies [4,5]. Thankfully, melanoma is at the vanguard of the personalized medicine movement [6,7], and the last 5 years have witnessed major therapeutic advances in melanoma research. Despite these advances, neither immune therapies nor cell growth inhibitors offer a truly optimal treatment as they fail to offer the ability to provide durable remission and well-tolerated side effects for the majority of patients. It is likely other therapeutic

strategies will be required for this complex disease.

Markers for metastasis

What drives melanoma cells to metastasize? This is a key question for clinicians and patients to answer. Clinico-pathological parameters can stratify patients according to risk of dissemination. However, there is a serious lack of effective markers, from either clinicians' or patients' perspective. When two patients exhibit the same Breslow thickness, the most powerful prognostic indicator of metastasis, we do not yet have the knowledge to be certain whether metastases will arise or not. This causes an unwelcome degree of uncertainty and anxiety, respectively. An improved understanding of the genetic and molecular basis of metastasis formation should provide important insights into the development of novel prognostic and therapeutic markers and go some way to fulfilling this unmet need.

Currently, no single protein marker reliably predicts the risk of metastasis.

KEYWORDS

- cell migration • chemotaxis
- lipid phosphate phosphatases
- metastasis • pagetoid spread
- signaling lipids

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In recent years, studies have investigated gene expression signatures based on mRNA levels in melanomas but interpretation is difficult due to translational and post-translational alterations, drawing into question the validity of the data *in vivo* [8]. It is not even clear if melanoma metastasis is driven by changes in tumor gene expression, as opposed to tumor size and environment. Given that metastasis is the most important cause of mortality, there remains an unmet need for a molecular marker of metastatic potential [9].

The role of LPA in driving invasion

We have recently described the role of extracellular breakdown of lysophosphatidic acid (LPA) in driving melanoma metastasis [10]. Through the direct visualization of chemotaxis *in vitro*, we demonstrated that the cells locally degrade LPA, generating a gradient from low in the tumor to high in the surrounding tissues. This gradient acts as a signal driving melanoma cells to migrate out of the tumor and invade. We showed that outward facing gradients of LPA are present across the invasive fronts of real melanomas *in vivo*. In addition to a downward invasion signal, these tumor-centric gradients provide a mechanism for the upward dispersal (pagetoid) spread commonly associated with invasive melanoma and also the lateral spread of radial growth phase melanomas and potentially in-transit metastases [11,12].

We also demonstrated that the greater the number of cells, the higher the rate of LPA depletion. These data suggest that there is a critical tumor density capable of generating a gradient sufficient to drive invasion. Below this density the tumor cannot generate a significant LPA gradient; above it a gradient is generated and a chemotactic signal results. Although depth of invasion is currently the best predictive marker for metastasis, there is evidence to support tumor volume as a better, albeit more challenging to calculate, predictive marker [13].

It is apparent that melanomas influence their immediate microenvironment by creating LPA gradients. This model dictates that, provided with a supply of chemoattractant and a high enough cell density to efficiently degrade the attractant, the cells will efficiently disperse. Therefore the source of LPA within the microenvironment and the growth of the tumor are likely to be the rate limiting factors in the dispersal of melanoma cells.

Are platelets the source of LPA?

Our data support melanomas as net depleters of LPA in their immediate microenvironment. If the melanoma cells are not producing the LPA then it would follow that there is an external source of LPA. It has been well documented that LPA is released from activated platelets as a result of inflammation [14,15]. There is growing evidence to support platelets contributing to the metastatic process, and Boucharaba *et al.* demonstrated the role of LPA in inducing bone metastases in mice injected with metastatic breast cancer cells [16–18]. This effect was mediated through LPAR 1. Interestingly, the cancer cells stimulated platelet activation and LPA release, and inhibiting platelets *in vivo* with the specific integrin $\alpha\text{IIb}\beta 3$ antagonist (Integrilin) produced a 70% fall in the concentration of circulating LPA and a 50% reduction in the extent of bony metastases. These results support local platelets as the source of LPA around the tumor.

Targeting platelets in patients with melanoma as a chemo-preventative strategy is therefore a subject that requires further study.

LPARs & LPPs as potential melanoma prognostic markers

We hypothesize that the presence of an LPA gradient is a marker for active chemotactic invasion and therefore may represent the much-needed prognostic marker for the risk of metastasis. Current classification systems for melanoma and even proposed improved systems combining genetic and morphological information do not include the microenvironment [19,20]. We believe that our proposed model is one of the most persuasive to supporting a role for a microenvironmental factor influencing the risk of melanoma metastasis. In order to develop a reliable prognostic marker for melanoma metastasis, it will be important to identify surrogate markers for outward LPA gradients due to the technical difficulty and cost associated with quantifying LPA gradients by mass spectrometry.

It will therefore be important to investigate the known key regulators of external LPA concentrations in the microenvironment – lipid phosphate phosphatases (LPPs) – and the receptors involved in LPA signal transduction (LPARs), of which there are at least five of each, and correlate their expression profiles and behavior *in vivo* with metastasis and Breslow thickness.

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Lipid phosphate phosphatases & LPA breakdown

Current thinking suggests that LPA is a simple, positive driver of tumor growth and spread. In this case metastasis should be favored by increased LPA production and/or decreased LPP expression, resulting in less breakdown and thus increased extracellular LPA [21,22]. Our data suggest the opposite – LPA gradients, not LPA concentrations *per se* appear to drive migration, so increased expression of LPP would act as a driver of metastasis. Using the gene expression-based tool generated by the Hoek group for predicting phenotype (the Heuristic Online Phenotype Prediction tool; [23]) there are statistically significant 1.8-fold and 1.9-fold increases in the expression of LPP1 (PPAP2A) and LPP3 (PPAP2B), respectively, in invasive compared with proliferative melanomas.

It is not known which of the LPPs are more important, although it may be the signature pattern of several LPP enzymes that is critical in determining the response. We therefore need to identify the key LPPs.

Targeting the LPA axis to inhibit invasion

Targeting cell motility to inhibit tumor cell invasion remains controversial as motility underpins such a range of normal biology. Similarly, it is unlikely that such anti-LPA cancer chemotherapy could be tolerated for any length of time as LPA homeostasis is essential for so many aspects of health. It will therefore be important to develop a deeper understanding of the role of the LPA axis in metastasis progression to identify alternative strategies to

blocking this pathway to avoid the unwanted side effects. Preliminary *in vitro* experiments inhibiting the LPPs have revealed that chemotaxis is abrogated. Targeting the enzymes that break down LPA, rather than those that synthesize it, may represent a more favorable approach.

Summary

We have shown that chemotaxis has a central, if not essential, role in the invasion of melanoma cells and LPA is a key driver of this response. We speculate that some aspects of the LPA axis may provide novel prognostic and therapeutic markers. The implication that the inflammatory response, and platelet activation in particular, play an essential role in melanoma progression is an area that requires particular attention.

These results challenge current concepts about how populations of cells migrate and invade, highlighting their ability to perform chemotaxis to self-generated gradients. There is now a great opportunity to study self-generated gradients – of LPA and other molecules – in not only melanoma, but other types of cancer cell in general.

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