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PRL-3/PTP4A3 phosphatase regulates integrin β1 in adhesion structures during migration of human ocular melanoma cells

Malika Foy[a,b], Océane Anézo[a], Simon Saule[a,b], Nathalie Planque[a,c,*]

[a] Institut Curie, PSL Research University, CNRS UMR3347/INSERM U1021, Orsay, France
[b] Université Paris Sud, Paris Saclay, Orsay France
[c] Université Paris Diderot, Sorbonne Paris Cité, Paris, France

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ABSTRACT

In a previous transcriptomic analysis of 63 ocular melanomas of the uvea, we found that expression of the PRL-3/PTP4A3 gene, encoding a phosphatase that is anchored to the plasma membrane, was associated with the risk of metastasis, and a poor prognosis. We also showed that PRL-3 overexpression in OCM-1 ocular melanoma cells significantly increased cell migration *in vitro* and invasiveness *in vivo*, suggesting a direct role for PRL-3 in the metastatic spreading of uveal melanoma. Here, we aimed to identify PRL-3 substrates at the plasma membrane involved in adhesion to the extracellular matrix. We focused on integrin β1, which is the most highly expressed integrin in our cohort of uveal melanomas.

We show that preventing PRL-3 anchorage to the plasma membrane i) abolishes PRL-3-induced migration in OCM-1 cells, ii) specifically enhances the spreading of OCM-1 cells overexpressing PRL-3, and iii) favors the maturation of large focal adhesions (FAs) containing integrin β1 on collagen I. Knockdown experiments confirmed integrin β1 involvement in PRL3-induced migration. We identified interactions between PRL-3 and integrin β1, as well as with FAK P-Y397, an auto-activated form of Focal Adhesion Kinase found in FAs. We also show that integrin β1 may be dephosphorylated by PRL-3 in its intracytoplasmic S/T region, an important motif for integrin-mediated cell adhesion. Finally, we observed that PRL-3 regulated the clustering of integrin β1 in FAs on collagen I but not on fibronectin.

This work identifies PRL-3 as a new regulator of cell adhesion structures to the extracellular matrix, and further supports PRL-3 as a key actor of metastasis in uveal melanoma, of which molecular mechanisms are still poorly understood.

1. Introduction

Dynamic protein, lipid, and carbohydrate phosphorylation play key roles in all cellular processes and tissue kinomes are now relatively well-characterized. In cancer, many kinases, which relay signals from the plasma membrane to the nucleus, become oncogenic when they are deregulated. The phosphatase families are much less well-studied, although they are equally important as they reverse kinase-mediated regulation. Several phosphatases have been shown to be deregulated in human cancers. We previously studied molecular deregulation of ocular melanoma of the uvea by high-throughput gene expression profiling of a cohort of 63 primary tumors after enucleation and found that the PTP4A3/PRL-3 gene (Protein Tyrosine Phosphatase 4A3/Phosphate Regenerating Liver-3) was more highly expressed in primary tumors at high risk of metastasis than in tumors at low risk [1]. The level of PRL-3 mRNA was also predictive of the metastatic risk. We further showed that PRL-3 overexpression in the human uveal melanoma cell line OCM-1 induced *in vitro* migration and *in vivo* invasion [1]. We currently investigate the molecular mechanisms deregulated by PRL-3 overexpression during uveal melanoma metastasis.

Intraocular melanomas can arise in three sites, the iris, ciliary body, or choroid, collectively referred to as the uveal tract. Uveal melanomas are highly aggressive tumors that preferentially develop macrometastases in the liver [2–4]. Patients die within two years from the time of metastatic diagnosis because of the lack of effective systemic treatment, underscoring the need to uncover the molecular mechanisms of uveal melanoma metastasis. Ocular melanomas are rare (mean-age adjusted worldwide incidence of 4.3 new cases per million each year [5]) and are much less studied than skin melanomas. Several high-throughput

Abbreviations: ECM, Extracellular matrix; FA, Focal adhesion; PTP, Protein Tyrosine Phosphatase

* Correspondence to: Institut Curie, Centre de Recherche, Centre Universitaire d'Orsay, Bâtiment 110, F-91405 Orsay Cedex, France.
E-mail address: nathalie.planque@curie.fr (N. Planque).

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analyses of large cohorts of uveal melanomas have been conducted at the genomic, transcriptomic, and proteomic levels during the last decade. The alterations are different from those of cutaneous melanoma. Driver point mutations for the initiation of the tumor were revealed, in more than 80% of the samples, in the GNAQ and GNA11 genes, which encode heterotrimeric G protein α-subunits [2–4]. Major alterations that are correlated with a high risk of metastasis are inactivating point mutations in the BAP1 (BRCA1 Associated Protein 1) gene, found in approximately 85% of metastatic tumors [2–4]. Other recently elucidated deregulated mechanisms in uveal melanoma have implicated MAPK and PI3K-AKT signaling pathways downstream activated heterotrimeric G proteins in tumor growth [2–4]. However, the mechanisms that regulate metastasis are still poorly understood. BAP1 knockdown in uveal melanoma cell lines inhibits melanocytic identity but does not increase cell migration and invasion [6].

Numerous published studies report the PTP4A3/PRL-3 gene to be frequently overexpressed in tumor samples from different origins, such as intestine, stomach, pancreas, lung, bladder, breast, ovary, prostate, and liver as well as in several malignant blood diseases [7,8]. These studies consist mainly of large cohorts of tumor samples in which mRNA levels were measured using high-throughput technologies or protein levels by immunohistochemistry. Mutations in the PTP4A3/PRL-3 gene have not yet been described by next generation sequencing analyses of cancer cohorts. Its expression level frequently correlates with metastatic risk and predicts poor patient survival [7,8]. Functional characterization of the PRL-3 protein in various cancer cell lines in culture and in murine metastasis models, either using overexpression or knockdown strategies, shows that PRL-3 promotes cell migration *in vitro* and metastatic invasion *in vivo* [7,8]. Its normal functions and tissue distribution are poorly characterized in mammals. The PRL-3 gene encodes a small dual specific phosphatase of 22 kDa, of the Protein Tyrosine Phosphatase (PTP) superfamily [7,8]. It contains a large catalytic domain and a carboxyterminal prenylation motif CAAX, similar to that found in the small GTPases of the Ras and Rho families. Prenylation anchors proteins to lipid membranes by post-translational addition of a long lipid chain (called prenyl) to the cysteine residue of the CAAX box by specialized cytoplasmic enzymes. PRL-3 can dephosphorylate tyrosine, serine, and threonine residues in proteins, as well as phosphoinositide lipids [7,8]. Several potential targets for membrane PRL-3 have been proposed following high-throughput phosphoproteomic or proteomic analyses, particularly proteins that makeup or regulate the cytoskeleton, such as ezrin, stathmin, keratin 8, cadherin CDH22, or NHERF1 [7–9]. All of these molecules may directly contribute to pro-migratory functions of membrane PRL-3 during metastatic spreading, due to their functions. Integrin β1 is another attractive candidate [10,11].

Integrins span the plasma membrane and attach different components from the extracellular matrix (ECM) to the actin cytoskeleton fibers [12–15]. During the migration of adherent cells, they provide traction points for the cell to crawl on the substratum [12,14,15]. The binding of integrins to the ECM induces them to cluster into macromolecular structures, which dynamically assemble and disassemble near the edge of the cell during migration [12,15]. Integrin clustering first induces the formation of small punctate structures called nascent Focal Adhesions (FAs) that either disappear or evolve into longer and larger mature FAs, due to more integrin clustering and the addition of new adhesome components, under appropriate conditions, for example when the cell pauses during migration [12,15]. FAs link actin fibers to integrin clusters through the assembly of many proteins, which are scaffold proteins, signaling effectors or regulators, such as kinases and phosphatases [12,15]. In addition to be cell-to-ECM contact points, FAs also constitute multiprotein signaling complexes in which one of the first downstream components to become activated by integrin clustering is FAK (Focal Adhesion Kinase) [12,16]. Several kinases are members of the adhesome and regulate subtle phosphorylation networks [12] (www.adhesome.org). Phosphatases are less well-understood. Reversible phosphorylation of a large number of FA components plays a key role in FA turnover, both for actin fiber linkage to the ECM, and for the activation of intracytoplasmic signaling pathways [14,17]. The binding of integrins to the ECM is highly regulated by phosphorylation of their intracytoplasmic tails [13,17]. Integrins are heterodimers formed by the non-covalent association of an α- and β-subunit [12–14,17]. Three intracytoplasmic phosphorylation motifs are found in β-subunits [13,14,17]. The motif found closest to the plasma membrane is the membrane proximal (MP)-NPxY motif, followed by the S/T region, and the membrane distal (MD)-NxxY motif (see the diagram in Fig. 4B). Phospho-mimetic and phospho-deficient mutations of these motifs in various integrins, including integrins β1 and β3, induced changes in cell shape, adhesion to the ECM *via* FAs, and migration in murine embryonic fibroblasts [17].

PRL-3 was found to interact with integrin β1 in co-immunoprecipitation experiments performed using protein extracts from human colorectal cancer cell lines [10,11]. The two tyrosine of the MP- and MD-motifs of integrin β1 seem to be dephosphorylated by PRL-3. Indeed, PRL-3 has been shown to dephosphorylate integrin β1 on the Y783 residue from the MP-NPxY motif in a GST pull-down assay *in vitro* [11]. PRL-3 overexpression and knockdown experiments in human colorectal cancer cell lines confirmed that the Y783 phosphorylation in integrin β1 was either more or less intense in the absence or presence of PRL-3, respectively [11]. PRL-3-induced dephosphorylation of the Y795 residue of the MD-NxxY motif may also occur *in vivo*, but the data are less convincing [11]. Integrin β1 mRNA is the most highly expressed integrin in our cohort of uveal melanomas, both in primary tumors at low or high risk of metastasis, as well as in liver metastases (Supplementary Fig. S6B, from the transcriptomic data set published in [1]). In this former study, we showed that uveal melanoma cells overexpressing PRL-3 migrate faster *in vitro*, and exhibit smaller FAs on collagen I than the control cells [1]. We thus reasoned that integrin β1 may be an important target of PRL-3 in uveal melanoma. In this study, we focused on cell-to-ECM contacts during cell migration. We propose that PRL-3, at the plasma membrane, acts as a regulator of integrin β1 in FA assembly and disassembly dynamics during cell migration, and that PRL-3 induces dephosphorylation of the S/T region of integrin β1.

2. Materials and methods

2.1. Plasmids, antibodies, ECM components, and drugs

The plasmids pEGFP-PRL3 and pEGFP-C104S were kindly provided by Qi Zeng (IMCB Institute, Singapore). The plasmid pcDNA3.1-EGFPc1 was purchased from Clontech, psi-mU6 shRNA anti-ITGB1-CMV mCherryFP from GeneCopoeia, and pCMV6-Entry ITGB1A-FLAG from Origene.

Mouse monoclonal anti-integrin β1 antibody (ab30394), rabbit polyclonal anti-integrin β1 (ab92671), and anti-integrin β3 (ab75872) antibodies were purchased from Abcam. Rabbit polyclonal anti-integrin β1 phospho-specific T788/T789 was purchased from Millipore (AB1929). Rabbit polyclonal anti-FAK total (sc-557), anti-p-FAK (Tyr397)-R (sc-11765-R), and mouse monoclonal anti-PRL-3 (318, sc-130355) were purchased from Santa Cruz Biotechnology. Mouse monoclonal anti-GFP Ab-2 was purchased from Thermo Scientific (clone GFP02). Mouse monoclonal anti-GFP (mixture of clones 7.1 and 13.1) was purchased from Roche. Mouse monoclonal anti-α-tubulin, anti-β-actin, and anti-FLAG M2 antibodies were purchased from Sigma-Aldrich. Anti-mouse and rabbit IgG secondary antibodies conjugated to Alexa Fluor (AF)–555 or AF-647 were purchased from Molecular Probes. DAPI (4′,6-diamidino-2-phenylindole, Sigma) was dissolved, diluted in PBS, and used at a final concentration of 0.1 mg/mL. Anti-mouse and anti-rabbit IgG HRP were purchased from Jackson ImmunoResearch.