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Commentary: "Alpha-enolase (ENO1) controls alpha v/beta 3 integrin expression and regulates pancreatic cancer adhesion, invasion, and metastasis"

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ABSTRACT

We have previously shown that in pancreatic ductal adenocarcinoma (PDA) cells, the glycolytic enzyme alpha-enolase (ENO1) also acts as a plasminogen receptor and promotes invasion and metastasis formation. Silencing of ENO1 in PDA cells induces oxidative stress, senescence and profoundly modifies PDA cell metabolism. Although anti-ENO1 antibodies inhibit PDA cell migration and invasion, little is known about the role of ENO1 in regulating cell-cell and cell-matrix contacts. We recently investigated the effect of ENO1 silencing on the proteome of PDA cells, and there was a significant downregulation of proteins involved in cell-cell and cell-matrix adhesion, including alphaV/beta3 integrin in ENO1-silenced PDA cells. These changes impaired the ability of ENO1-silenced cells to adhere to collagen I and IV and fibronectin, and caused an increase in RGD (tripeptide Arg-Gly-Asp)-independent adhesion to vitronectin (VN) via urokinase plasminogen activator receptor (uPAR). Binding of uPAR to VN triggers integrin-mediated signals, which results in ERK1/2 and Rac activation, accumulation of ROS (Reactive Oxygen Species) and senescence. In ENO1-silenced cancer cells, the use of an anti-uPAR antibody led to reduced ROS production and senescence, and an increase in cell apoptosis. Overall, a decrease of in vitro and in vivo cell migration and invasion of ENO1-silenced PDA cells was observed. This commentary summarizes new data demonstrating that ENO1 promotes PDA survival, migration and metastasis by cooperating with integrins and uPAR. These data represent a springboard for a novel therapeutic strategy to counteract PDA progression based on combined targeting of integrins, uPAR and ENO1.

Background

The involvement of alpha-enolase (ENO1) in cancer progression has become increasingly more addressed and described in recent years. Disease-related roles of ENO1 are mostly attributed to its immunogenic capacity, DNA-binding ability and plasminogen receptor function[1]. In particular, ENO1 overexpression has been associated with cancer proliferation and invasion, and correlated with poor clinical outcomes[2,3]. The role of ENO1 in pancreatic ductal adenocarcinoma (PDA) has been reported in the literature by our previous studies; firstly we identified a correlation between ENO1-circulating antibodies in the blood and a better response to therapy and survival of PDA patients[4,5]; we then defined ENO1 as a tumor-associated antigen that induces both humoral and T cell-specific responses in PDA patients[6]; finally we found an association of ENO1 with the promotion of PDA tumor invasion due

to its role as a plasminogen receptor, by promoting matrix degradation through plasminogen activation[7]. Moreover, we demonstrated that targeting ENO1 using passive immunotherapy with anti-ENO1 antibodies showed good results in terms of reducing metastasis and extending survival in vivo[7]. In addition, DNA-vaccination against ENO1 in a mouse model obtained similar results[8]. Finally, ENO1 has been found to be one of the leading regulators of the Warburg effect and plays an important role in carcinogenesis and tumor maintenance[9]. It is known that inhibition of glycolysis prevents cancer development as it is essential for proliferation, invasion and metastasis of cancer[10]. Analysis of silenced ENO1 in PDA cells revealed a profound modification in their metabolism, which was associated with an increase of oxidative stress and senescence[9]. Starting from this evidence, the following study was carried out to highlight the key role of ENO1 in pancreatic cancer, with particular focus on the regulation of cell-cell and cell-matrix contacts and functions.

Our results

In the study "Alpha-Enolase (ENO1) controls alpha v/beta 3 integrin expression and regulates pancreatic cancer adhesion, invasion, and metastasis"[11], we characterized the role of ENO1 as a multifunctional protein involved in migration and invasion in PDA by exploiting a knockdown using shRNA. Using a combination of atomic force microscopy and confocal microscopy, we were able to observe strong morphological changes in shENO1 PDA cells, especially showing an increase in surface roughness due to the loss of cytoskeleton organization. The reorganization of cytoskeleton proteins suggested that the absence of ENO1 caused impairment of the ability of PDA cells to adhere to the ECM (extracellular matrix) and migrate.

Liquid chromatography- Tandem Mass Spectrometry (LC-MS/MS), a semi-quantitative proteomic analysis, was previously performed on ENO1-silenced and control CFPAC-1 pancreatic cancer cells. Compared to control CFPAC-1 cells, ENO1-silenced cells showed significantly altered expression of 17 proteins involved in cell adhesion and cytoskeleton organization, which was further confirmed by PCR analysis[9]. In particular, the proteins: actin-related protein 2/3 complex subunit 4 (ARPC4), F-actin-capping protein subunit alpha2 (CAPZA2) and breast cancer anti-estrogen resistance protein 1 (BCAR1), usually associated with actin remodeling and poor prognosis in cancer[12-15] were up-regulated after ENO1 silencing. Paradoxically, the increase in these proteins was not associated with increased aggressiveness of pancreatic cancer, as ENO1-silenced cells showed a reduced ability to grow and invade both in vivo and in vitro. Further analysis of the post-transcriptional modifications of these proteins after ENO1 silencing could help to clarify this discrepancy. Instead, other proteins generally associated with poor prognosis, such as Galectin 3 (LGALS3), family with sequence similarity 129 member B (FAM129B), integrin subunit alpha V (ITGAV) and MUC5AC (Mucin 5AC)[16-19] or those that augmented metastasis, such as Ahnak Nucleoprotein (AHNAK), Anterior Gradient 2 (AGR2), PDZ and LIM domain 2 (PDLIM1), Galectin 4 (LGALS4), Golgi Glycoprotein 1 (GLG1) and Serpin Family B Member 5(SERPINB5)[20-25] were down-regulated in PDA cells. Of note, the interaction of some of these proteins with cytoskeleton elements is critical for cell shape modifications: PDLIM1 forms complexes with a-actinin1 and 4, thus regulating cell invasion and metastasis formation; LGALS3 interacts with β-catenin increasing tumorigenesis; and catenin delta 1 (CTNND1) interacts with E-cadherin promoting cell spreading[26]. Other down-regulated proteins were proposed as targets for PDA cancer therapy, such as ITGAV and AGR2, or as early biomarkers like MUC5AC and LGALS4[27,28]. In accordance with the proteomic data[9], we observed that silenced ENO1 PDA cells showed a decreased ability to adhere to the ECM (with the exception of vitronectin (VN), and a reduction in invasion and migration, suggesting a central role for ENO1 in metastasis spreading. Among all the proteins, we focused on integrins that are involved in the cell-ECM interaction. Silencing of ENO1 caused an increase of alpha5/beta1 and a decrease of alphaV/beta3 integrins. Fibronectin (FN) is recognized by both complexes, which cooperate in promoting cellular attachment and spreading[29] with distinct roles: alpha5/beta1 integrins determine adhesion strength through their catch-bond binding to FN, whereas alphaV/beta3 integrins mediate force-induced reinforcement of the adhesion site through their connection with the actin cytoskeleton[30]. We hypothesized that even in the presence of alpha5/beta1 integrins, which start the adhesive process, the absence of alphaV/beta3 in ENO1-silenced PDA cells impairs the reinforcement signals to establish strong contacts with the matrix and migrate. Interestingly, alphaV complexes with different subunits (beta1, beta3, beta5, beta6 and beta8) mediate adhesion to the most common ECM proteins, such as FN, collagens (COLs) and VN[31]. Consequently, down-regulation of alphaV in ENO1-silenced PDA cells is one of the major triggering factors for the decrease in their binding to ECM, with the exception of binding to VN, which actually increases. In cancer, VN interacts with members of the integrin family (alphaV/beta1, alphaV/beta3, alphaV/beta5 and alphaIIb/beta3) through the RGD motif[32]. As alphaV and beta3 subunits were down-regulated in ENO1-silenced cells, all of the above-mentioned complexes cannot be considered responsible for the VN-increased adhesion of shENO1 cells; this led us to hypothesize that a non-integrin receptor is involved in the binding of VN. Urokinase Plasmin Activator Receptor (uPAR) binds the N-terminal SMB domain on VN and, as the binding site is different from that integrins, usually the two groups of proteins bind VN independently, but cooperate for

intracellular signaling[32,33]. uPAR lacks a cytosolic domain but is able to send downstream signals by associating with trans-membrane integrins (mainly beta1 and beta3), even independently from direct integrin/matrix interaction in a ligand-independent manner[33]. In this study, we demonstrate how, in the absence of ENO1, uPAR is up-regulated and is responsible for the strong interaction between cells and VN, even in the absence of binding of integrins. Moreover, the absence of beta3 with a concomitant expression of beta1 integrin causes an unbalance in the intracellular signaling, leading to activation of proto-oncogene tyrosine-protein kinase Src and extracellular signal-regulated kinases (ERK1-2), and p38 mitogen-activated protein kinase (MAPK) inactivation. The ERK signal provided by uPAR–beta1 integrin interaction is required for activation of the small signaling GTPase subfamily Rac[34]. Taken together, these signals drive the cells to a senescence status, impairing tumor progression as well as apoptosis. Interestingly, we also found that, in the absence of ENO1, targeting of uPAR efficiently prevents ROS production and senescence while promoting apoptosis, suggesting that a combinatory strategy to simultaneously target ENO1 and uPAR could be effective to inhibit PDA tumor progression and invasion.

Future perspectives

Overall, we demonstrated a high dependency of PDA cells on components of the integrin family to exert their spreading capability. While we clearly showed that ENO1 is essential in mediating this effect, many other points remain to be addressed and investigated in future studies; a deeper investigation of additional targets identified from our proteomic screening could define other pathways dependent on ENO1. Moreover, from a clinical perspective, it is clear that ENO1 represents an optimal hit for targeted therapies. Particularly, the strong effects we demonstrated in reducing tumor spreading could be combined with other therapies targeting uPAR, inhibiting the intracellular signaling of integrin beta1/beta3, or inhibiting the binding between cells and ECM promoting cell death through anoikis. Additional investigations could include addressing transcription regulation mediated by ENO1, in order to unravel possible transcription factors correlated to the phenotypes seen in the knock-down cells. In this view, alterations in the signaling pathways in the cells could lead to epigenetic remodeling that is worth investigating in future studies.

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