



Pancreatic cancer cell invasion: mesenchymal switch or just hitchhiking?

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For the majority of pancreatic cancer patients, metastatic spread is the most life-threatening issue, significantly shortening survival (1). Yet, the road for a cancer cell to successfully set its metastatic niche and to grow there is fortunately long and sown with pitfalls. Consistently, the estimated time for pancreatic cancer patients to develop a widely disseminated disease through subclonal metastatic evolution from a parental clone inside the primary carcinoma is 6.8-year, as recently reported (2). To the important question of whether the poor prognosis of pancreatic cancer is due to its late diagnosis, or because it metastasizes early during clonal evolution, Yachida *et al.* indeed answered that there is a long latency to development of an infiltrating cancer, and thus a large window of opportunity for early diagnosis and cure. Yet, until early detection of pancreatic cancer becomes routine, the reality is that most patients will likely continue to be diagnosed with advanced disease, as recently mathematically predicted through a comprehensive study that benefited from a large group of patient's autopsy data (3).

Subclonal metastatic evolution implies gain of (epi)genetic heterogeneity that may arise from preexistent small populations of cancer stem cells that continuously give rise to genetically diverse populations of non-tumorigenic cells (4), as lately evidenced in pancreatic cancer (5). As epithelial-to-mesenchymal transition (EMT) seems a robust feature that arises during pancreatic cancer progression (6,7), even if recently reconsidered (8), the probability of generating, through the EMT program, highly tumorigenic cells capable of self-renewal and of repopulation of all phenotypic progeny present within the neoplasm (9), is

plausible. The EMT is a reprogramming phase giving to epithelial cells not only features of stemness, but above all of motility and invasiveness, properties required to successfully accomplish their metastasis route. Mesenchymal traits are acquired at the expense of epithelial markers which are lost through transcriptional and/or epigenetic repression. Whether this phenotypic switch is irreversible, or subjected to constant reversibility considering that both epithelial and mesenchymal markers have been found in a same cell, is under intense debate (10). Oversimplification would be dangerous and one imagines that the picture is not unique, since (I) the high cellular and molecular heterogeneity of pancreatic tumor confers robust plasticity; (II) the stresses that pancreatic tumors are facing during their metastatic cascade or disease treatment history (e.g., stroma-derived, mechanical, hypoxia, genotoxic) select the most adapted subclones, and EMT seems a prerequisite. Nevertheless, the epithelial phenotype of metastases has shed the light on the reverse transition, i.e., mesenchymal-to-epithelial transition (MET), putatively occurring in the metastatic cancer cells that may have previously lost their epithelial phenotype to acquire metastatic skills, MET giving them back their proliferative properties required for efficient metastatic outgrowth.

EMT is driven by a family of transcription factors, mainly of ZEB, SNAIL and TWIST families, that in a multi-stage process remodel epithelial cell architecture, for the acquisition of increased cell migration, invasion and survival. Lineage tracing experiments in genetically engineered mouse models (GEMMs) have revealed that EMT occurs early during the pancreatic disease evolution (7). Indeed, circulating tumor cells with acquired mesenchymal traits

enabling their migration/invasion into the blood flow, yet incapable of establishing metastases, are delaminated from the primary neoplasm as soon as precancerous lesions are present. Nevertheless, two recent studies on breast and pancreatic cancer suggested that EMT, although critical for tumor cells to acquire chemoresistance features, is dispensable for a tumor to metastasize (8,11). Those studies reopened the debate on the need “to be mesenchymal or not” during the cell metastatic cascade. Although Zheng *et al.* upon conditional deletion of the transcription factors *twist* or *snail* genes in a pancreatic cancer GEMM background, still observed as many metastases in those conditions as in the parental model, with metastatic cells keeping their epithelial features (E-cadherin expression) (8), one can envision that, in this model, partial EMT is present owing to compensatory mechanisms driving mesenchymal switch. Consistently, other transcription factors have been reported to do the job.

Recently, Heeg *et al.* reported that the expression of the ETS-transcription factor ETV1, which is essential for mesenchymal differentiation and stromal tissue identity during pancreatic branching morphogenesis, is progressively induced during the pancreatic ductal carcinogenesis sequence from preinvasive [pancreatic intraepithelial neoplasia (PanIN)] to invasive/metastatic lesions (12). Then, they showed that ETV1 is critical for stromal expansion and metastatic progression of pancreatic tumors. To do so, they used pancreatic ductal cells isolated from invasive lesions in GEMM (Pdx1Cre;*Kras*^{G12D/+}; *p53*^{fl/+}, named KPC), that could be traced owing to the expression of reporter proteins (YFP or dTomato), and in which they overexpressed ETV1. Orthotopic graft of ETV1-overexpressing cells in immunodeficient mice increased, as compared to parental mock cells, primary tumor growth *via* stroma expansion, and metastasis development. Interestingly, ETV1 overexpression provided to epithelial cells mesenchymal and metastatic features, in association with loss of E-cadherin expression, with upregulation of the matricellular protein SPARC expression, and with deposition of hyaluronic acid. The regulation by ETV1 was transcriptional, through direct activation of *sparc* and hyaluronic synthase 2 (*bas2*) promoter activities. Interestingly, SPARC was expressed in YFP-positive tumor cells, suggesting a direct molecular link with ETV1. *sparc* knockout in ETV1-overexpressing KPC cells functionally abrogated their potential, upon orthotopic graft in mice, to form stroma-rich tumors and to metastasize (although some SPARC was still detectable in the host stroma), placing SPARC as an autonomous functional target of ETV1 pro-metastatic effect in cancer cells. Altogether,

these results place the transcription factor ETV1 as a novel driver of mesenchymal traits in pancreatic tumor cells, and tumor cell-derived SPARC as its direct functional effector. Whether this transcription factor is indeed a master regulator of stroma expansion and metastasis driven by KPC cells that endogenously present a 184-fold increase of ETV1, as compared to normal pancreatic ductal cells, is currently under investigation using conditional invalidation of *etv1* in the KPC background. First results confirm that ETV1 is indeed involved but not as dramatically as expected, suggesting that activation of other pathway(s) may compensate for ETV1 absence. Unraveling one mechanism for hyaluronic acid production in pancreatic tumors (via HAS2 regulation by ETV1) is of high therapeutic interest since this matrix glycosaminoglycan is responsible for mechanic stresses that result in vascular collapse and subsequent reduced perfusion of these tumors (13). Enzymatic strategies aimed at alleviating hyaluronic acid to restore perfusion and help the diffusion of chemotherapies to tumor cells, are under clinical trials.

Redundancy of transcription factors that regulate the epithelial versus mesenchymal fate is multiple, suggesting that selection pressure during the subclonal metastatic evolution is high to keep this plasticity feature in the selected cancer clones. Among the panoply of transcription factors regulating EMT, the two major isoforms of the paired-related homeodomain transcription factor 1 (PRRX1), PRRX1a and PRRX1b, have recently been uncovered to play a role (14). Those transcription factors are up-regulated during ductal development, induction of acinar-to-ductal metaplasia, and evolution of PanINs. Strikingly, those splicing variants have opposite effects, i.e., EMT for PRRX1b (induction of invasion and tumor dedifferentiation), and MET for PRRX1a (stimulation of metastatic outgrowth in the liver and tumor differentiation), giving a first mechanistic information (through alternative splicing regulation between both isoforms) for the dynamic switch occurring between EMT and MET, observed in primary tumors *vs.* in metastases, respectively. The hepatocyte growth factor (HGF) was identified as a novel transcriptional target of PRRX1b explaining at least in part its mesenchymal-inducing effect, and therapeutic targeting of HGF in combination with gemcitabine was given as a promising strategy in a preclinical model of pancreatic cancer. Lastly, the transcription factor RUNX3 has been identified when comparing the expression profiles of non-metastatic versus metastatic pancreatic tumor cells that presented, in a KPC

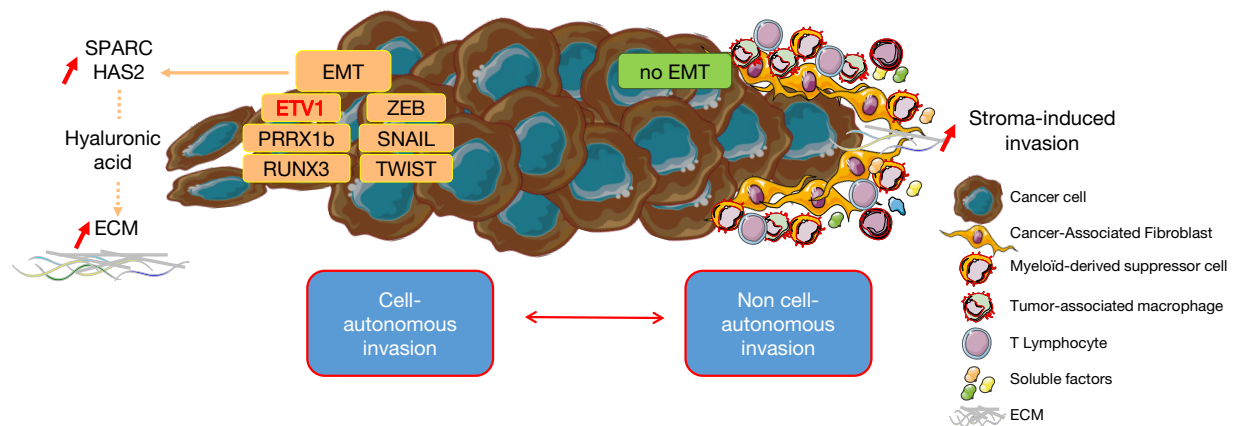


Figure 1 Tumor cell invasion may involve either cell-autonomous events depending on partial or complete EMT, or non-cell-autonomous features including tumor cell hitchhiking on the stroma [e.g., cancer-associated fibroblasts (CAFs) (22)]. Whereas transcription factors of the ZEB, SNAIL or TWIST families have been well-described in EMT, more recently described factors including ETV1 allow, in addition to repression of epithelial markers (E-cadherin), the direct transcription of genes that encode for ECM or that control ECM production (SPARC, hyaluronic acid) (12). HAS2, hyaluronin synthase 2; ECM, extracellular matrix; EMT, epithelial-to-mesenchymal transition; PRRX1, paired-related homeodomain transcription factor 1.

background, a mono-allelic loss of *smad4/dpc4*, versus wild-type or *smad4/dpc4* bi-allelic loss, respectively (15). High RUNX3 expression explained cell differences in metastatic skills, mainly because RUNX3 can control both extracellular matrix (ECM) protein production (e.g., osteopontin, SPARC) and/or proliferation, depending on *smad4/dpc4* status, facilitating cell seeding and/or growth in the primary tumor and metastatic niches. This study is consistent with the two phenotypes of pancreatic cancer patients that differ not in their morphologic differences at diagnosis but in their metastatic efficiencies for which inactivation of *SMAD4*, and as a result loss of SMAD4 protein expression, is a marker (16). Surprisingly in this study, metastatic potential *in vivo*, and migration or invasive skills *in vitro*, were not reminiscent of E-cadherin expression loss, yet a conventional marker of EMT. These results suggested that loss of E-cadherin expression is not a sufficient marker to monitor “complete functional EMT”, and that cell invasion and metastasis can co-exist with persistence of the epithelial phenotype (e.g., high E-cadherin expression).

Consistently, the induction of EMT with downregulation of E-cadherin expression is likely tunable, dependent on whether complete or partial EMT signaling is present. The EMT program is thought to be controlled by the tumor microenvironment, including by soluble factors released by stromal cells [e.g., cancer-associated fibroblasts

(CAFs) (17,18) or tumor-associated macrophages (19,20)], mechanical (tumor stiffening) or metabolic stresses (hypoxia) (21), which locally downregulate epithelial characteristics and facilitate cell escape from the primary tumor. However, with local upstream signaling lost, cells undergo MET reversion after metastatic seeding in the secondary organ (10). Yet likely indispensable during the metastatic process, the observed differences in EMT reprogramming, [i.e., induced in a cell-autonomous fashion through irreversible mesenchymal phenotype acquisition, or stroma-induced tunable and localized phenotypic switch (e.g., in leading cells of the invasive fronts)], may explain also the large differences observed in cell migration, individual in undifferentiated tumors, or collective in differentiated tumors where CAFs certainly help epithelial cancer cell clusters to crawl into the stroma (22) (Figure 1). These types of migration eventually co-exist in different regions of a same tumor, and are plastic such as during therapeutic stresses, probably explaining the difficulty to therapeutically target the metastatic process (23).

Nevertheless a common feature of cancer invading cells is their entwined required relationship with the stroma, that they may either directly produce through EMT reprogramming, as described by Heeg *et al.* (12), or interact with during collective yet highly efficient migration in which EMT may not be necessary.

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References

1. Yachida S, Iacobuzio-Donahue CA. Evolution and dynamics of pancreatic cancer progression. *Oncogene* 2013;32:5253-60.
2. Yachida S, Jones S, Bozic I, et al. Distant metastasis occurs late during the genetic evolution of pancreatic cancer. *Nature* 2010;467:1114-7.
3. Haeno H, Gonen M, Davis MB, et al. Computational modeling of pancreatic cancer reveals kinetics of metastasis suggesting optimum treatment strategies. *Cell* 2012;148:362-75.
4. Magee JA, Piskounova E, Morrison SJ. Cancer stem cells: impact, heterogeneity, and uncertainty. *Cancer Cell* 2012;21:283-96.
5. Li C, Heidt DG, Dalerba P, et al. Identification of pancreatic cancer stem cells. *Cancer Res* 2007;67:1030-7.
6. Iacobuzio-Donahue CA, Fu B, Yachida S, et al. DPC4 gene status of the primary carcinoma correlates with patterns of failure in patients with pancreatic cancer. *J Clin Oncol* 2009;27:1806-13.
7. Rhim AD, Mirek ET, Aiello NM, et al. EMT and dissemination precede pancreatic tumor formation. *Cell* 2012;148:349-61.
8. Zheng X, Carstens JL, Kim J, et al. Epithelial-to-mesenchymal transition is dispensable for metastasis but induces chemoresistance in pancreatic cancer. *Nature* 2015;527:525-30.
9. Mani SA, Guo W, Liao MJ, et al. The epithelial-mesenchymal transition generates cells with properties of stem cells. *Cell* 2008;133:704-15.
10. Diepenbruck M, Christofori G. Epithelial-mesenchymal transition (EMT) and metastasis: yes, no, maybe? *Curr Opin Cell Biol* 2016;43:7-13.
11. Fischer KR, Durrans A, Lee S, et al. Epithelial-to-mesenchymal transition is not required for lung metastasis but contributes to chemoresistance. *Nature* 2015;527:472-6.
12. Heeg S, Das KK, Reichert M, et al. ETS-Transcription Factor ETV1 Regulates Stromal Expansion and Metastasis in Pancreatic Cancer. *Gastroenterology* 2016;151:540-553.e14.
13. Provenzano PP, Cuevas C, Chang AE, et al. Enzymatic targeting of the stroma ablates physical barriers to treatment of pancreatic ductal adenocarcinoma. *Cancer Cell* 2012;21:418-29.
14. Reichert M, Takano S, von Burstin J, et al. The Prrx1 homeodomain transcription factor plays a central role in pancreatic regeneration and carcinogenesis. *Genes Dev* 2013;27:288-300.
15. Whittle MC, Izeradjene K, Rani PG, et al. RUNX3 Controls a Metastatic Switch in Pancreatic Ductal Adenocarcinoma. *Cell* 2015;161:1345-60.
16. Blackford A, Serrano OK, Wolfgang CL, et al. SMAD4 gene mutations are associated with poor prognosis in

- pancreatic cancer. *Clin Cancer Res* 2009;15:4674-9.
17. Moatassim-Billah S, Duluc C, Samain R, et al. Anti-metastatic potential of somatostatin analog SOM230: Indirect pharmacological targeting of pancreatic cancer-associated fibroblasts. *Oncotarget* 2016. [Epub ahead of print].
 18. Duluc C, Moatassim-Billah S, Chalabi-Dchar M, et al. Pharmacological targeting of the protein synthesis mTOR/4E-BP1 pathway in cancer-associated fibroblasts abrogates pancreatic tumour chemoresistance. *EMBO Mol Med* 2015;7:735-53.
 19. Harney AS, Arwert EN, Entenberg D, et al. Real-Time Imaging Reveals Local, Transient Vascular Permeability, and Tumor Cell Intravasation Stimulated by TIE2hi Macrophage-Derived VEGFA. *Cancer Discov* 2015;5:932-43.
 20. Karnevi E, Rosendahl AH, Hilmersson KS, et al. Impact by pancreatic stellate cells on epithelial-mesenchymal transition and pancreatic cancer cell invasion: Adding a third dimension in vitro. *Exp Cell Res* 2016;346:206-15.
 21. Lamouille S, Xu J, Derynck R. Molecular mechanisms of epithelial-mesenchymal transition. *Nat Rev Mol Cell Biol* 2014;15:178-96.
 22. Gaggioli C. Collective invasion of carcinoma cells: when the fibroblasts take the lead. *Cell Adh Migr* 2008;2:45-7.
 23. Friedl P, Alexander S. Cancer invasion and the microenvironment: plasticity and reciprocity. *Cell* 2011;147:992-1009.

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