



p-21 activated kinase 4 (PAK4) role in pancreatic cancer stem cells

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Comment on: Tyagi N, Marimuthu S, Bhardwaj A, *et al.* p-21 activated kinase 4 (PAK4) maintains stem cell-like phenotypes in pancreatic cancer cells through activation of STAT3 signaling. *Cancer Lett* 2016;370:260-7.

Abstract: On January 28, 2016 *Cancer Letters* published an article entitled “p-21 activated kinase 4 (PAK4) maintains stem cell-like phenotypes in pancreatic cancer cells through activation of STAT3 signaling”, which demonstrates that PAK4 regulates pancreatic cancer stemness through STAT3 activation. Particularly expressed in a subpopulation of cancer stem cells (CSCs) co-expressing CD24, CD44 and EpCAM, PAK4 is associated with enhanced levels of transcription factors inducing pluripotency, self-renewal and dedifferentiation properties such as OCT-4/NANOG/SOX2 and KLF-4. Additionally, PAK4 could be inducing resistance to gemcitabine treatment in this subpopulation of cells. Although previous studies observed an association between PAK4 and pancreatic cancer or PAK4 and CSCs, the data here presented are novel demonstrating first experimental evidence of PAK4 in pancreatic cancer stemness. However, experimental strategy is still very limited as conclusion has been drawn by using exclusively two different *in vitro* cell lines. Importantly, a broader *in vitro* testing and further clinical approach is required before clinical utility as novel therapeutic target can be claimed.

Keywords: Cancer stem cells (CSCs); chemoresistance; p-21 activated kinase 4 (PAK4); pancreatic cancer; stemness

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Pancreatic Ductal Adenocarcinoma (PDAC) is a dismal disease with 5-year survival ranking from 1.2% to 5% and being the fourth leading cause of cancer-related death in the United States (1). It is expected to become the second cause of cancer death by 2030 (2). This poor prognosis can be explained by late diagnosis and treatment resistance including radio/chemo and immunotherapy modalities (3). Consequently, numerous efforts are being made and should continue on both strategies: (I) finding non-invasive biomarkers for early detection and (II) searching efficient treatment that could avoid disease progression and metastasis.

Based on recent publications, one of the entities contributing to the highly tumorigenic, rapid progression, metastasis and therapy-resistance are the cancer stem cells (CSCs) (4-7). Although increased importance has been given to CSC and their contribution to PDAC pathogenesis,

molecular mechanisms controlling the stemness and further clinical implications are not yet clarified.

For some years now, investigators around the world have tried to identify biomarkers that could help in the selection and targeting of specific population of CSC. However, numerous attempts to define pancreatic CSC-specific biomarkers and their associated functions have been challenging (8). So far, only the presence of molecules as CD24, CD44, CD133, ALDH1 and EpCAM and pluripotency-associated genes such as OCT3/4, SOX2 and NANOG have been clearly associated to pancreatic CSC (4, 9,10). Still, in order to achieve clinical applicability mechanisms explaining its function and regulation must be investigated.

The results recently published in *Cancer Letters* by Tyagi *et al.* (11) offer a new insight of PAK4 involvement in the maintenance of the stem cell-like phenotype in PDAC.

Here, the authors demonstrate that p-21 activated kinase 4 (PAK4) is specifically overexpressed in pancreatic CSC and, through STAT3 activation induces sphere formation capacity and chemoresistance to gemcitabine.

PAK4 is a serine/threonine kinase characterized as downstream effectors of Rac and Cdc42 (12) and important player in several cellular processes including progression of cancer (13-15) including pancreatic cancer (16). Additionally, in different types of tumors but not PDAD, PAK4 has been also associated to CSC (17).

The study presented by Tyagi *et al.* includes data from only two different pancreatic cancer cells lines which express high levels of PAK4 (MiaPaCa and T3M34). The authors defined a CD24+/CD44+/EpCAM+ subpopulation of each cell line is defined as CSC and those CD24-/CD44-/EpCAM- as non-CSC and observed higher levels of PAK4 in CSC compared with non-CSC. With this bases, the study provides evidence of a reduction in CSC markers CD24, CD44, EpCAM and also ALDH1 after silencing PAK4 in both studied cell lines. Additionally, PAK4 silencing leads to a reduction of stemness-associated transcription factors KLF4, SOX2, NANOG and OCT4 at mRNA and protein level and decreased sphere forming ability as indication of self-renewal potential and undifferentiated state. By using growth assays, Tyagi *et al.* showed that PAK4 silenced cells were more sensitive to gemcitabine compared with control cells.

This interesting and pioneer study must be taken with precaution since results comes from only two cell lines. Overall, data must be confirmed in broader systems including primary cell lines and cells from patient's tumor. Future experiments comparing results obtained in cells already described as harboring high levels of CSC (i.e., L3.6) with those possessing low levels (i.e., PaTu8988t) will provide a better idea of PAK4-STAT3 role in pancreatic cancer.

Regarding to CSC biomarkers-identification, discussion could be open when authors define CD24+/CD44+/EpCAM+ subpopulation as CSC and CD24-/CD44-/EpCAM- as non-CSC. Since ALDH1 and CD133 are also markers for pancreatic CSC we recommend providing information about PAK4 expression in ALDH1+ and CD133+ cells. Additionally, PAK4 expression levels of subpopulations being only positive for one or two of these markers are not presented here. Furthermore, based on their results it is difficult to asseverate that PAK4 is the unique responsible for showed effect in CSC. A comparative effect on cells with high and low PAK4 levels should be demonstrated.

Regarding the mechanism associated to these CSC properties, the study investigated the involvement of STAT3 in PAK4-induced stemness in pancreatic cancer

concluding that transcriptional activity of STAT3 promoter is decreased upon PAK4 silencing. More in detail, STAT3 proteins goes to the nucleus in high PAK4 expressing cells compared with those harboring low levels. Significant reduction in STAT3 phosphorylation was observed in PAK4-silenced MiaPaCa and T3M4 cells. Accordingly, STAT3 overexpression restores transcriptional activity of SOX2, NANOG and OCT4. Moreover, sphere formation capacity is also restored after forced activation of STAT3 in PAK4-silenced cells.

PAK4-STAT3 cross-talk is the mechanistic discovery of this work and contributes to the knowledge of pathways that could be essential for pancreatic cancer development. Here, although western-blot and luciferase assay methodology used to demonstrate PAK4-STA3 link is valuable, immunofluorescence technique could have provided better and more elegant evidence of nuclear translocation.

Finally, in this manuscript, authors show that PAK4-silenced cells are more sensitive to gemcitabine as compared with control cells. In fact, this data support recent results published by Moon *et al.* demonstrating that PAK4 is a predictive marker of gemcitabine sensitivity in pancreatic cancer cell lines (18). They claim that resistant cell lines (Capan-2, PANC-1, and SNU-410), knockdown of PAK4 by siRNA resulted in restoration of sensitivity to gemcitabine. Probing effect in sensitive and resistant cell lines to gemcitabine would add value to the work.

A pertinent question that could arise here is whether this effect could be observed when Abraxane is administrated in combination with gemcitabine (novel regimen for pancreatic cancer patients).

Importantly, it would be very interesting to elucidate the role of PAK4 in epithelial-to-mesenchymal transition (EMT) process. Numerous publications linked CSC to EMT in various solid tumors including PDAC. Although evolutionary concept of CSC properties and functions is still controversial, various cellular processes give us evidence of its importance and complexity and EMT is contributing greatly on this.

Although authors claim their results support clinical utility as therapeutic target, further validations must be made. Indeed, one of the progresses toward the development of novel therapeutic approaches could pass through PAK4 inhibition. However, technical difficulties for *in vivo* procedures and lack of available drugs, makes mandatory further and deep basic and translational investigations.

Furthermore, in order to continue with more translational studies, expression of PAK4 in human pancreatic tissues could be evaluated to elucidate whether any correlation exist with histological subtype, differentiation status or response

to chemo and radiotherapy treatment. Finally, future directions should be taken to clarify if PAK4-STAT3 could have significance with clinical applicability in liquid biopsy.

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Footnote

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