REPORT
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DYRK1A INHIBITION AS A STRATEGY FOR REMODELLING THE TUMORAL STROMA AND SENSITISING THE IMMUNOTHERAPY BASED ON IMMUNE CHECKPOINT INHIBITORS IN PANCREATIC CANCER

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1. Summary and results

Pancreatic ductal adenocarcinoma, abbreviated as PDAC, is the most common form of pancreatic cancer. Pancreatic tumors are highly aggressive and are characterized by intense desmoplastic reaction. The relationship between the tumor's reactive microenvironment and the malignant cell defines an immunosuppressive environment that correlates with tumor progression and low response to immunotherapy. Our groups demonstrated that the kinase DYRK1A plays a protumorigenic role in PDAC by promoting signaling in malignant cells through the c-MET and EGFR receptors. In this study, we have investigated the role of DYRK1A in cancer-associated fibroblasts (CAFs) and explored the effects of DYRK1A inhibition on malignant cells and/or CAFs on the tumor microenvironment.

We have observed that DYRK1A plays a relevant role in the migration of CAFs as its inhibition, either by genetic or pharmacological means, impairs CAFs migration. Reduction of DYRK1A expression in CAFs leads to transcriptomic alterations that affect important cellular pathways such as TGF-β signaling or collagen biosynthesis among others. A reduction in the contractile capacity of CAFs is also observed, which could be attributed to a reduction in their elasticity as confirmed by atomic force microscopy experiments. Overall, we believe that DYRK1A inhibition in CAFs may be causing changes in the cellular cytoskeleton that confer a more myofibroblastic phenotype with tumor-retaining activity. Furthermore, DYRK1A inhibition in CAFs reduces the migratory phenotype of cancer cells, suggesting that DYRK1A may be regulating soluble factors produced by CAFs involved in this process.

Manipulation of DYRK1A kinase levels in PDAC cancer cell lines or CAFs has revealed its influence on the expression of a subset of secreted proteins. These include extracellular matrix proteins like collagens and proteoglycans, as well as factors such as cytokines, growth factors, and growth factor receptors present in the extracellular environment. Particularly, several of these dysregulated proteins identified in both cell types have been implicated in modulating the PDAC tumor microenvironment. Among the proteins identified in the DYRK1A-dependent secretome, we have validated a decreased secretion of key cytokines from malignant cells and CAFs, which could contribute to reducing the tumor's immunosuppressive environment. Alterations in these proteins may explain observed paracrine cellular phenotypes, such as the reduction in

macrophage migration resulting from DYRK1A inhibition in malignant cells. On the other hand, the inability to form tumor organoids or to impair their growth in co-culture conditions with CAFs with inhibited DYRK1A may be related to the role of certain modulated cytokines in maintaining a tumor stem cell phenotype.

Additionally, we have also demonstrated a synergistic effect of DYRK1A inhibition and some of the chemotherapy agents currently in clinical use in PDAC. This finding reinforces their anticancer effects and suggests a potential therapeutic strategy for PDAC. Finally, during the course of this project, we identified a relevant molecule, forming complexes with DYRK1 proteins, with a role in regulating the dynamics of microtubules in pancreatic cancer cells.

2. Relevance and potential future implications

- Inhibiting DYRK1A **could mitigate metastasis.** Reduction of CAF migration upon DYRK1A inhibition could mitigate the metastatic behavior of pancreatic cancer cells. Additionally, the impaired migration of tumor cells in the presence of conditioned media from CAFs with inhibited DYRK1A would support a role of DYRK1A in metastasis.

- Inhibiting DYRK1A would **modulate the progression of PDAC** by acting on the tumor microenvironment.

- Inhibiting DYRK1A would **reduce the tumor's immunosuppressive environment**.

- Inhibiting DYRK1A has a **synergistic effect** with some current chemotherapeutic **treatments** and could contribute to an improvement in immunotherapy.

- DYRK1A could be a promising **therapeutic target** for PDAC.

3. Scientific bibliography

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