Supression of Snail and Slug to avoid EMT in pancreatic adenocarcinoma

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Research Proposal

Background

The epithelial-mesenquimal transitions (EMT) are essential for the tumor growing and the metastasi process[1]. In cancer progression, EMT seems to promote the spread of cells from the tumor mass and facilitates tissue invasion by the regulation of matrix metalloproteinase production and alternate cytoskeleton organization[2]. Many activators of this transition are transcription factors that repress the expression of E-cadherin, such as Snail and Slug [3]. In this research, we propose to inhibit the expression of Slug and Snail to inhibit metastasis of pancreatic adenocarcinoma.

Hypothesis

If we repress the expression of Snail and Slug, we will also repress the epithelial-mesenquimal transition. If EMT is not possible, it will inhibit the metastatic process in pancreas adenocarcinoma cells. We will use human pancreas adenocarcinoma cell lines as a model, as well as a subcutaneous pancreatic cancer xenograft tumor model induced in mice or tumor intrapancreatic injections in mice. We will foccus in the NF-κB-Snail-RKIP pathway.

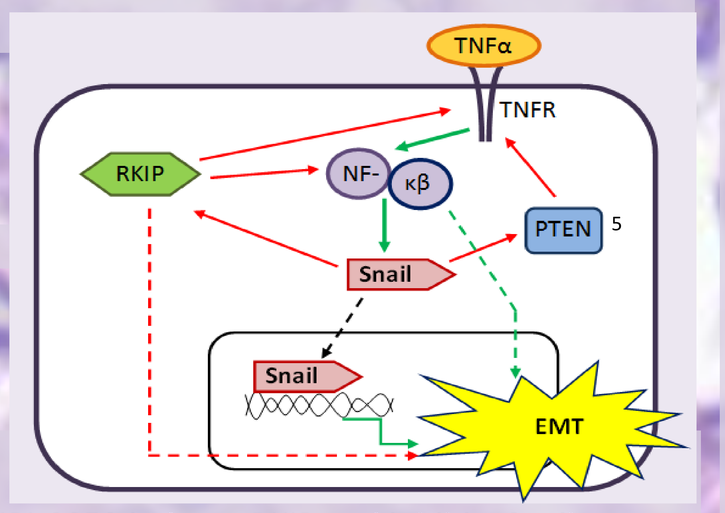

Figure 2. *NF-κB-Snail-RKIP pathway*

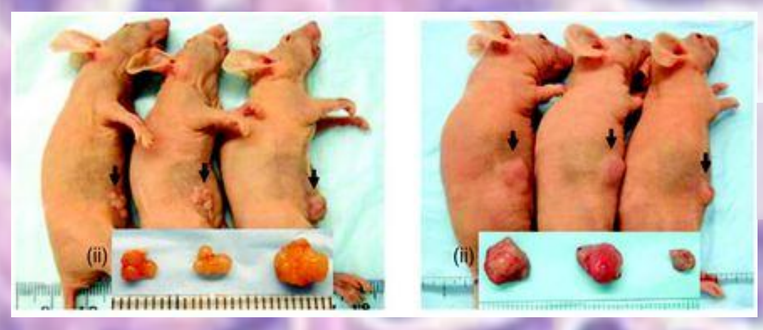

Figure 1. *NUDE mice with induced pancreas adenocarcinoma*[4]

Specific aims

1. The proteasome inhibitor NPI-0052 induces RKIP and inhibits Snail.
2. Nitric Oxide Inhibits Tumor Cell Metastasis via Dysregulation of the NF-κB/Snail/RKIP Loop.
3. (-)-Epigallocatechin 3-gallate inhibits Snail by regulating HDAC activity.
4. Inhibiting Interactions of Lysine Demethylase LSD1 with Snail/Slug Blocks Cancer Cell Invasion.

Methods

- Real-time PCR: RKIP transcription levels in cells.
- Weastern Bloot Analysis: Protein expression and genic products indication.
- Protein Assays: Total level of protein in a solution.
- EMSA: Protein–DNA or protein–RNA interactions.
- Clonogenic Assays: Effectiveness of agents on cell survival and proliferation.
- Different *Kits* are explained in the experimental.

Experimental

Specific aim 1

- NPI-0052 treatment in human pancreas adenocarcinoma cell lines
- NPI-0052 treatment and radiation to NUDE mice, with either AsPC-1 xenographt or intrapancreatic injection *in vivo*
- NPI-0052 inhibits the NF-κB promoter activity

Specific aim 2

- DETANONOat produces cellular fenotip reversion
- DETANONOat inhibits SNAIL expression
- Inhibition of DNA binding activity by Snail
- Direct role of Snail supression in EMT inhibition
- NO as a mediator in mesenquimal fenotip reversion from mice cells with intrapancreatic injection or carrying MiA-PaCa2 and AsPC-1 xenographt

Specific aim 3

- EGCG effect in cell death
- HDAC activity Assay

Specific aim 4

- Parnate effect in Slug/LSD1 interaction and E-cadherina promoter repression dependent on Slug
- E-cadherina expression in Parnate treated cells
- Parnate effect in migration, invasion and pancreas adenocarcinoma cell lines proliferation

Animals and human cell lines Models

- Subcutaneous pancreatic cancer xenograft tumor model or intrapancreatic tumor injection in NUDE mice (AsPC-1 o MiA-PaCa2)
- Human pancreas adenocarcinoma cell lines (AsPC-1 cell line)

Research group

- Researcher 1 *(FTJ)*
- Researcher 2 *(FTJ)*
- Researcher 3 *(FTJ)*

Timetable of the project

Project	2014			2015			2016		
1.Proteosome NPI-0052									
2. Nitric oxid (NO)									
3.Epigallocatechin 3-gallate									
4. Parnate									
Writing articles from each point						1,2	1,2	3	4

Budget

	Year 1	Year 2	Year 3	Total
Personal	18.000	18.000	18.000	54.000
Research costs (€)				
Equipment	15.000	10.000	5.000	30.000
Consumables Animals Cultures	18.000	18.000	18.000	54.000
Fieldwork/Travel		2.000	5.000	7.000
Total	51.000	48.000	46.000	**145.000**

Societal significance

Cancer is an illness that has a high impact on the humanbeing in our society. This high impact promotes the advancement of essential research for cures to this illness. This research proposal is focused on pancreas adenocarcinoma, which has a bad prognosis and has no treatment. We will try to inhibit EMT in this type of cancer with this research. We expect to obtain promising results to treat patients suffering from pancreas adenocarcinoma.

Possible patentability

Invention background: Inhibition of Snail and Slug expression to inhibit the pancreas adenocarcinoma metastasis.

Methods and Novelties: Use of proteosome inhibitor NP-0052, NO and Parnate; never used before in pancreas adenocarcinoma.

Aplication: Inhibition of EMT in pancreas adenocarcinoma and, consequently the metastasis process.

"Potential Claims"

1. Snail and Slug inhibition in pancreas adenocarcinoma
1.1 Use of proteosome NP-0052 in pancreas adenocarcionma as a Snail inhibitor
1.2 NO in pancreas adenocarcionma as a Snail inhibitor
1.3 EGCG in pancreas adenocarcionma as a Snail inhibitor
1.4 Parnate treatment in pancreas adenocarcionma as a Snail inhibitor
1.5 Snail inhibition in pancreas adenocarcionma

References

1. Kalluri R, Weinberg R. (2009). The basics of Epithelial-Mesechymal transition. *J Clin Invest*. 119(6):1420-1428. (19)
2. Zhang K, et al. (2011). Slug enhances invasion ability of pancreatic cancer cells through upregulation of *matri*metalloproteinase-9 and actin cytoskeleton remodeling. *Lab Invest*. 91(3):426-438
3. Hotz Birgit. (2007). Epithelial to Mesenchymal Transition: Expression of the Regulators Snail, Slug, and Twist in Pancreatic Cancer. *Clin Cancer Res*. 13; 4769
4. Huang C., et al. (2012). A Novel FoxM1-Caveolin Signaling Pathway Promotes Pancreatic Cancer Invasion and Metastasis. *Cancer Res 72;*655
5. García de Herreros, A. et al. (2008) Repression of PTEN Phosphatase by Snail1 Transcriptional Factor during Gamma Radiation-Induced Apoptosis. *Mol Cell Biol*. 28(5): 1528-1540

Background picture: *pancreatic adenocarcinoma*