



## Intracellular responses triggered by solid lipid nanoparticles in prostate cancer cells PC-3: Evaluation of the TGF- $\beta$ pathway

Fernanda G. Fóssa\*, Carolina Cassago, Marcelo B. de Jesus.

### Abstract

The use of nanoparticles holds promising new applications in medicine. Among the nanoparticles, Solid lipid nanoparticles (SLN) are noteworthy because they are produced with biodegradable and biocompatible components. Several studies have shown that cells can internalize and process SLN, and much has been studied about their therapeutic effects. Surprisingly, little research has investigated how SLN affect intracellular signaling pathways. The study of endocytosis associated with signaling is indispensable to understand the impact of these nanoparticles on eukaryotic cells. Previous results from our laboratory showed a correlation between SLN internalization and galectin-8 translocation into the nucleus of prostate cancer cells (PC-3). Galectin-8 is related to the activation of the TGF- $\beta$  signaling pathway. This pathway is deregulated in advanced stages of cancer, increasing cell migration and invasion. Here, we evaluate the effects of SLN on the TGF- $\beta$  signaling pathway. Uncovering the underlying mechanisms that may be triggered by nanoparticles in cancer cells may be helpful to develop and make use of safer nanomedicine tools.

### Key words:

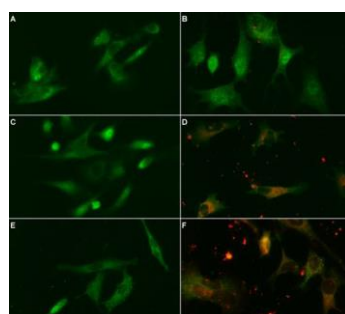
Intracellular signaling, solid lipid nanoparticles, TGF- $\beta$

### Introduction

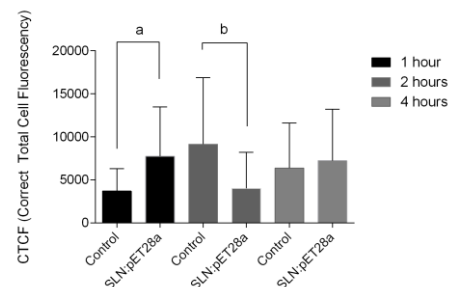
The organism development relies on a coordinated regulation of the intercellular processes. This process can also suffer influence from the signals shared between the cells, which will trigger specific signaling pathway, like TGF- $\beta$  pathway. In canonical pathway, after the activation of TGF- $\beta$  membrane receptors, Smad2/3 proteins are phosphorylated and translocated to the nucleus. There, phospho-Smad2/3 acts as a transcription factor, stimulating apoptosis and cycle cell arrest in normal cells. In cancer cells, TGF- $\beta$  activation can stimulate chemoattraction, migration, invasion, and metastasis<sup>1</sup>. Previous results from our laboratory indicate that SLN translocates galectin-8, a TGF- $\beta$ 's pathway stimulator, to the nucleus within 2 hours. Therefore, our aim was to evaluate whether SLN are capable of activating TGF- $\beta$  pathway. In addition, we investigated the influence of charge, concentration, and the consequences for cell metabolism.

### Results and Discussion

PC-3 cells were transfected with SLN for 1, 2, and 4 hours and prepared for immunofluorescence. Labeling with Phospho-Smad2/Smad3 (Cell Signaling) primary antibody (Image 1) and further analysis of the nuclear fluorescence (Image 2) demonstrate that TGF- $\beta$  pathway can be activated by the SLNplex. When TGF- $\beta$  pathway is activated in cancer cells, this pathway can induce cell invasion, growth, and metastasis<sup>2</sup>.



**Image 1.** Phospho-Smad2/Smad3 (Cell Signaling) in PC-3 cells during transfection by SLN:pET28a fixed with 1 hour (A-B), 2 hours (C-D), and 4 hours (E-F) of treatment, in absence (A, C and E) or presence (B, D and F) of SLN:pET28A.



**Image 2.** Analysis of fluorescence intensity of Phospho-Smad2/Smad3 relative to Image 1. a – 1 hour control vs SLN:pEt28a and b – 2 hours control vs SLN:pEt28a  $p < 0.05$  ANOVA, Tukey's test.

### Conclusions

Solid lipid nanoparticles are capable of activating TGF- $\beta$  pathway in PC-3 cells, now the biological relevance on cellular metabolism will be explored.

### Acknowledgement

We thank the members of Nano-Cell Interactions Lab for all the assistance, PIBIC/CNPq for the financial support.

<sup>1</sup> Massagué, J. *Nat Rev Mol Cell Bio* **2012**, *13*, 10.

<sup>2</sup> Massagué, J. *Cell* **2008**, *134*, 215-230.