Characterization of genetically modified mouse prostate organoids

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1. Abstract

Prostate cancer (PCa) is the second most common cancer in men worldwide and the risk of developing this malignancy increases with age. Previous research has shown that Teratocarcinoma-Derived Growth Factor 1 (TDGF1, Cripto) is associated with PCa progression. Cripto is a fetal oncoprotein that affects the regulation of stem cell differentiation, embryogenesis and tissue growth, and remodeling. If misregulation of Cripto occurs, it can lead to the development and progression of cancer, such as PCa. The results showed that the organoids transduced with luciferase, expressed bioluminescence. The transduction of Cripto by means of a Lentiviral construct showed that the organoid cells expressed more Cripto.

2. Introduction

Prostate cancer

Prostate cancer (PCa) is the second most common cancer in men worldwide, and the risk of developing this disease increases with age. PCa is an epithelial tumor that develops from the epithelial cells of the prostate gland. The prostate cells express androgen receptors (AR), which are stimulated by testosterone to promote cell proliferation. There are several treatment options for localized PCa. Prostatectomy is considered the standard treatment. If PCa is diagnosed at an advanced and metastatic stage, palliative therapy such as hormone therapy or chemotherapy may be used. [1]

The fetal oncoprotein Cripto

Therapy for primary tumors is effective, but unfortunately tumor recurrence and bone metastases still occur in a significant proportion of patients. Therefore, there is interest in finding molecular mediators that influence this progression. Cripto is a fetal oncoprotein that influences the regulation of stem cell differentiation, embryogenesis, and tissue growth and remodeling. If misregulation of Cripto signaling occurs, cancer development and progression can occur, as has been shown for breast cancer or PCa. [2]

Organoids

Organoids are three-dimensional (3D), self-assembling organotypic cell structures that proliferate from adult stem cells. Working with organoids has many advantages as this system recapitulates the heterogeneity of tumors. Traditional two-dimensional (2D) in vitro cell culture models are unable to replicate the complex microenvironment of a tumor. In contrast, 3D cell structures such as organoids resemble real human organs, are in some cases histologically nearly identical, and are also better suited to mimic in vivo tumor physiology. [3]

3. Aims and leading Questions

To generate luciferase-expressing organoid lines as a model for in vivo tracking of cancer cells.
→ Is it possible to introduce luminescence into the organoid model and exploit this tool to perform in vivo tracking?

To generate mouse prostate organoids carrying genetic modifications such as overexpression of Cripto.
→ Can mouse prostate organoids be genetically edited and prostate cell`s phenotype changed by overexpressing Cripto?

References
[1] Takeda, 2021, para. 10; ONKO Internetportal, 2018, para. 1-2
[2] Balcioglu et. al., 2020, p. 1; Rangel et. al., 2012, p. 1
[3] Kretzschmar, 2020, p. 501-503; Kim, Koo & Knoblich, 2020, p. 571-572; Zundert, Fortuni & Rocha, 2020, p. 1-2
[4] Wang et.al., 2009, pp. 495-500

Figures
Figure 1: Buchicchio, L. Bern: own figure
Figure 2: Buchicchio, L. Bern: own figure

4. Methods and material

Same procedure for transduction of organoids with luciferase as well as for Cripto.

- Plasmid extraction
- Production and equipping of the lentivirus with the plasmid
- The organoids are cultivated in 2D
- Transduction of the organoid cells with the plasmid
- Cultivation of the organoid cells in 3D → Organoids

Normal mouse prostate organoids of the strain “B6;129S-Nkx3 1tm4(cre/ERT2)Mms/Nci X B6;129-Gt(ROSA)26Sortm2Sho/J” were used. [4]

5. Results

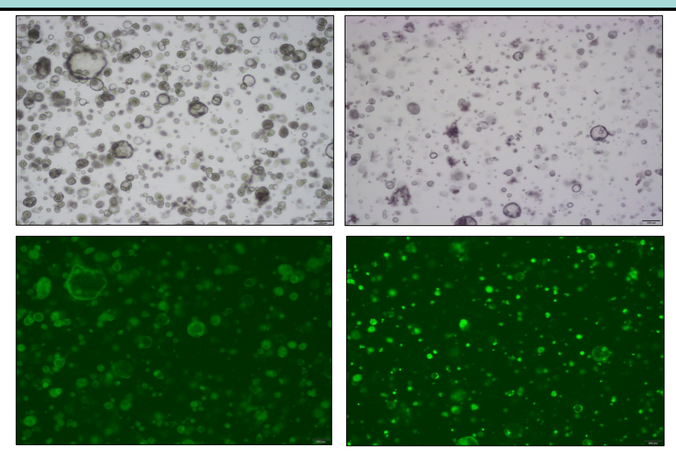

Figure 1: The images on the right are the control organoids. The images on the left are N. mouse prostate organoids with luciferase

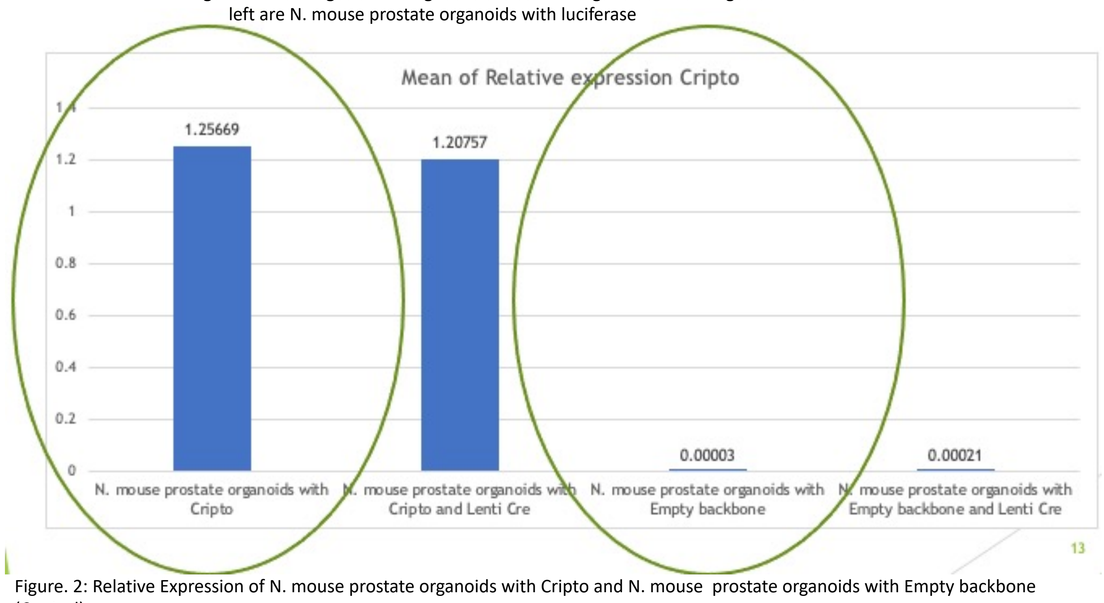

Figure. 2: Relative Expression of N. mouse prostate organoids with Cripto and N. mouse prostate organoids with Empty backbone (Control)

6. Discussion and conclusion

Luciferase-positive organoids

Based on the images acquired by fluorescence microscopy (see figure 1), the control organoids showed a lower signal. These data suggest that transduction of luciferase was successful in normal prostate organoids. It shows that genetic modification of mouse organoids is possible.

Cripto-positive organoids

Based on the qPCR results (see figure 2), a clear difference was observed between the relative expression of normal mouse prostate organoids with Cripto and the organoids with the empty backbone. This means that the transduction with Cripto was successful.

Outlook

Future work will attempt to characterize the aggressive behavior of genetically engineered organoids by injecting fluorescently labeled carcinogenic mouse prostate organoids into the heart of mice and then tracking them using bioluminescence. This approach could enable the identification of new therapeutic targets and lead to the discovery of new biomarkers that could provide effective treatment for PCa.