DDX3X role characterisation in colorectal cancer:



An oncogene or tumour suppressor?



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Bachelor's Degree in Biomedical Sciences (2022-2023)

1. Research proposal elaboration

This final degree thesis has consisted of the **design** of a research proposal on a relevant topic in the current biomedical research field.

Methodology

- Revision of reviews and original articles found in Pub ed
- Searches in web pages of providers of kits, reagents and consumables for reaearch.

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Aims To choose the most suitable method for each research question To plan an experimental design for a research proposal

Keywords

Colorectal cancer, DDX3X, dual-role protein, β -catenin, Snail, targeted therapy.

2. Introduction

The prognosis of **colorectal cancer (CRC)** is currently unsatisfactory, with high rates of relapse and subsequent metastatic spread. Thus, new combinatorial targeted therapies are needed to accomplish **long-term control of CRC**.

DDX3X is a **dual role protein** that has oncogenic and tumour suppressor described roles in CRC by enhancing β -catenin stability and inhibiting Snail, respectively.

Snail/E-cadherin and Wnt/ β -catenin pathways are interconnected and involved in the initiation of epithelial to mesenchymal transition (EMT) in CRC, a process that has been associated with increased rate of cancer recurrence and decreased survival of CRC patients.

Therefore, DDX3X emerges as a potential target for the treatment of CRC. However, the context in which DDX3X expresses each role needs to be clarified to determine which patients could **benefit from a DDX3X inhibitor therapy**.

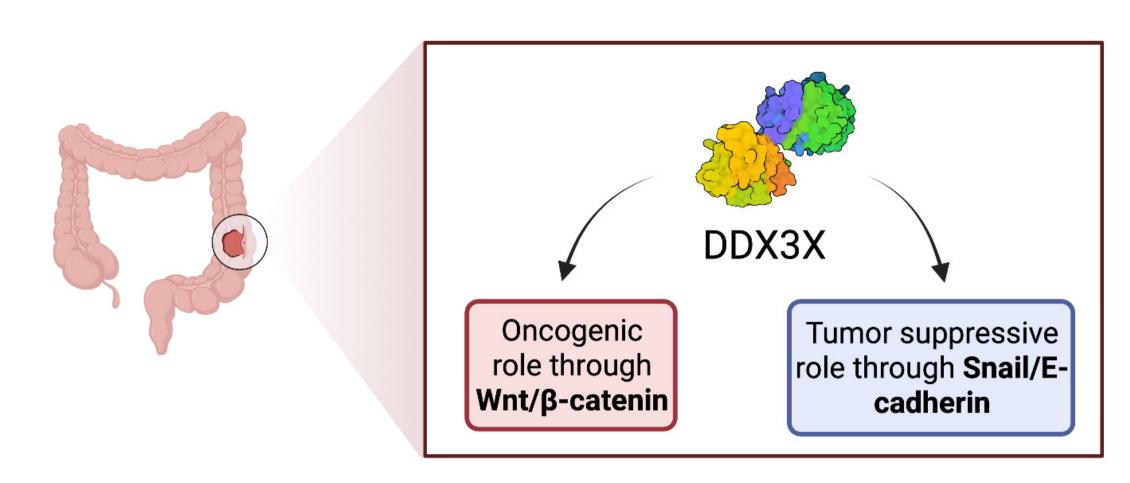


Figure 1. DEAD-Box RNA helicase-3 X-linked (DDX3X) as a dual role protein in CRC.

3. Aims & Hypothesis

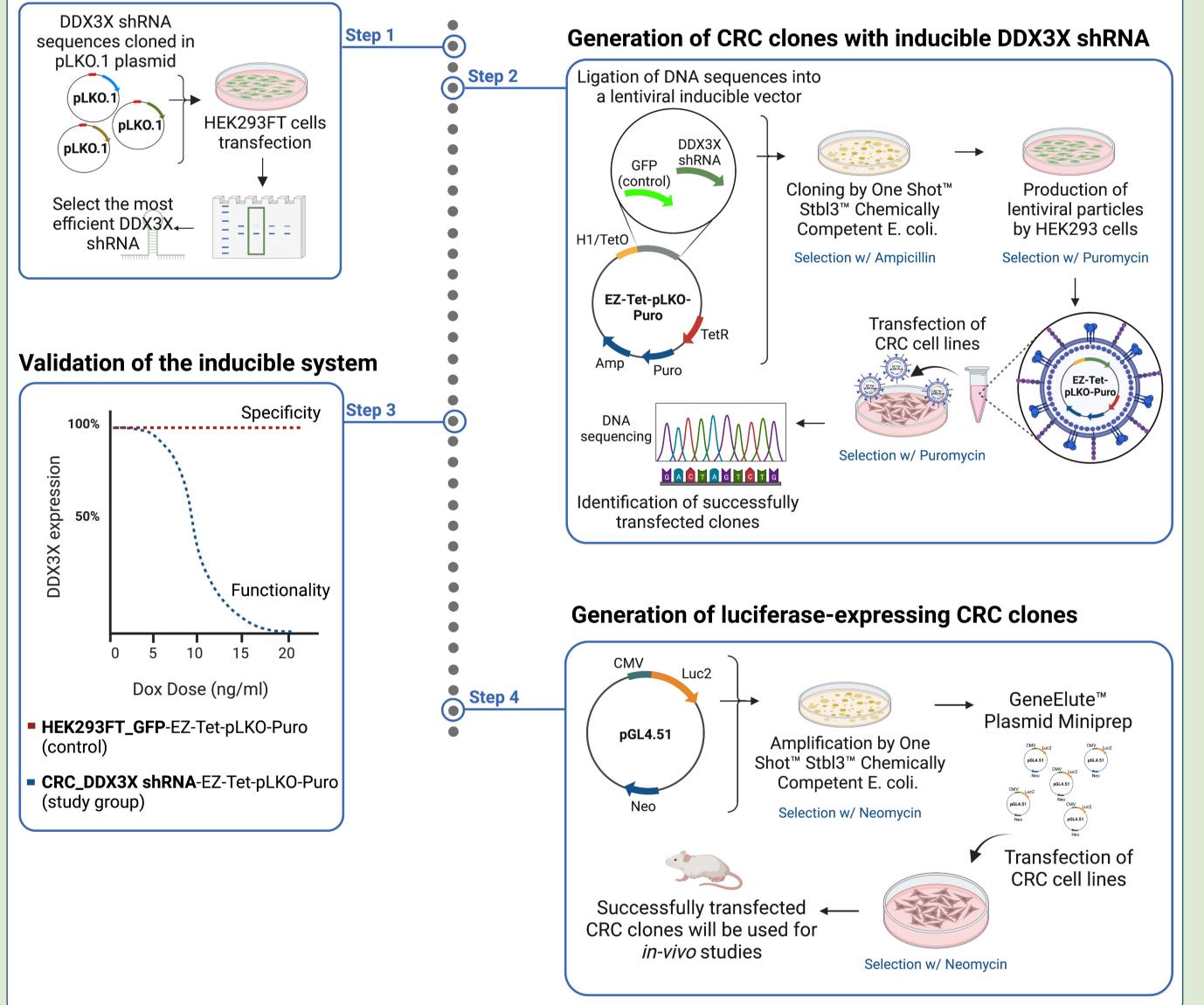
Given that DDX3X oncogenic role has only been described in Adenomatous Polyposis Coli (APC)-wild-type CRC cells, and 70% of sporadic CRCs emerge from APC inactivating mutations, the aim of this work is to **investigate the role of DDX3X in CRCs harbouring APC mutations** in Wnt/β-catenin signalling pathway.

It is hypothesized that in CRCs with this genetic background DDX3X can express its tumour suppressor role through the repression of Snail, thus excluding these patients from the treatment with a DDX3X inhibitor.

4. Methods. Generation of CRC clones with inducible expression of a DDX3X shRNA

A lentiviral vector containing an inducible shRNA will be prepared to *knock-down* DDX3X expression in HCT116 cell line (control) and DLD-1, SW480, and Colo205 cell lines (which harbour APC-mutations).

Validation of a DDX3X silencing system



6. Expected results

- It is expected to find out if the mutational status of APC can help to distinguish which patients can benefit from a treatment with a DDX3X inhibitor in combination with other targeted therapies.
- If DDX3X acts as a tumour suppressor through Snail inhibition in CRCs with APC loss, as hypothesised, in 70% of sporadic CRCs DDX3X expression might be beneficial.

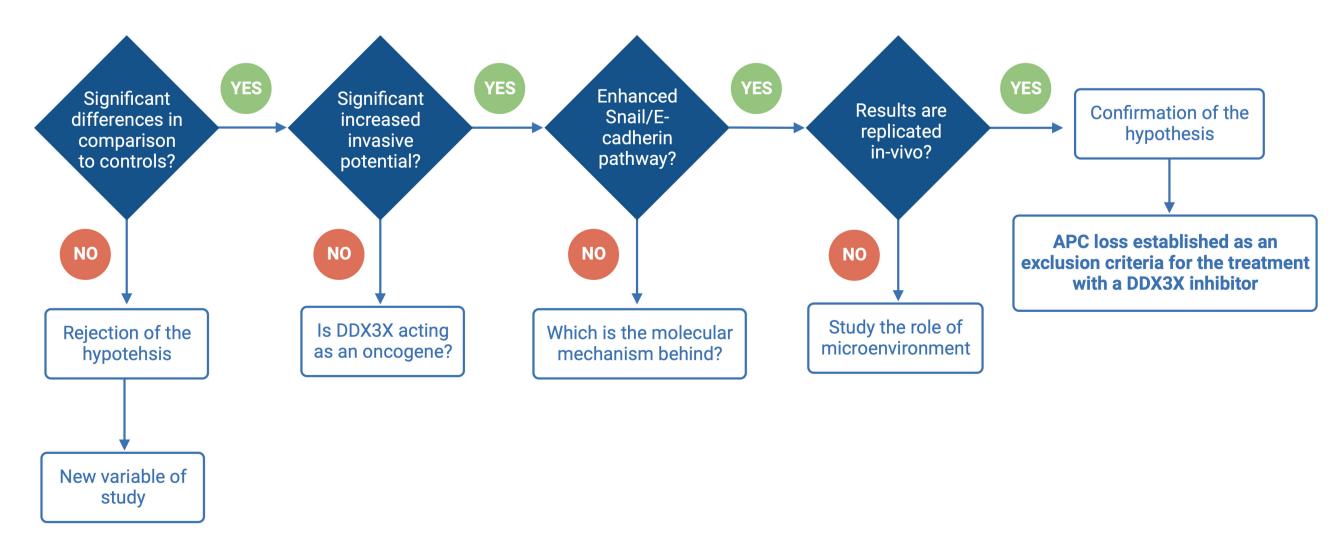
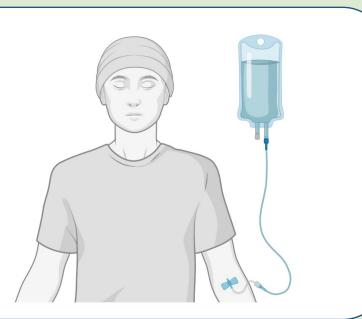


Figure 3. Flow chart of the possible results in DDX3X knock-down group of APC-mutated cell lines (study group).

7. Relevance of the study

Translational application → Establishing inclusion/exclusion criteria for the treatment with a DDX3X inhibitor will allow the election of the proper combinatorial therapies for CRC patients (personalized treatment).



5. Workflow 1. DDX3X role in-vitro From Jan to Aug Determining the role of DDX3X in (2024)**APC-mutated CRCs Control group** 1.1 Assess DDX3X basal levels by qPCR and Western Blot 1.3 Proliferation assay **APC-wt** CRC cell line 1.2 Create a DDX3X *knock-down* clone for each cell line **HCT116** Transfection of **CRC** cell lines **Study group** EZ-TetpLKO-Puro **APC-mut** CRC cell lines Immune detection of BrdU incorporated Establishment of into newly synthesized DNA succesfully SW480 Colo25 transfected clones 1.4 Transwell migration/invasion If there are problems with the DDX3X knockdown system, CRISPR-Cas9 knock-out If no significant differences observed, a new variable of study could be set Invaded cells Invasive Index = Migrated cells 2.2 Transcriptomic and proteomic validation **Proteome Discoverer** of significant hits HRP-polymer Software to detect the 2.1 Bottom-up proteomics most significant hits 2. DDX3X molecular Secondary Ab mechanism in-vitro Primary Ab Elucidating the molecular **Antigen** mechanisms behind DDX3X in APC-mutated cancers. Copies per reaction From Sep to Feb **Immunohistochemistry** Liquid chromatography/Tandem mass Western Blot (2024-2025)aRT-PCR (subcellular localisation) (quantification) spectrometry analysis 3.1 Generate Luc-expressing cells If in-vitro results are not CRC cells transfection replicated in-vivo, the role of 3. DDX3X characterisation in-vivo with pGL4.51 microenvironment on DDX3X function will be assessed. From Dec to Dec Evaluation of DDX3X role in APC-mutated CRCs (2024-2025)using an in-vivo orthotopic model. 3.4 In-vivo procedures 3.2 Generation of two CRC orthotopic models 3.5 Proliferation assay 3.7 Invasion assay in NOD.Cg-Prkdc^{scid} II2rg^{tm1WjI}/SzJ mice n= 8 mice/group (male/female) Submucosal injection **Detection of EMT-**Circulating Tumor Cells in the distal rectum Blood samples by Flow Citometry collection 3.3 Generation of DDX3X knock-down groups for APC-wt and APC-mut CRCs Bioluminiescence 3.8 Detection of in-vitro imaging validated markers in Water supplemented 3.6 Metastasis assay with doxycycline tumor samples **Experimental endpoint** tumor volume 1cm³ If differences between sexes are If failing to generate orthotopic reported, the study would be tumors in mice, CRC cell lines with replicated in-vitro from cells better engraftment can be ordered derived from male/female patients.

Figure 2. Worflow schematic representation including the timings and proposed contingency plans in some critical steps.

8. Diffusion plan 9. Financial e

• Publication in Oncology Journals with high impact factor. THE LANCET COCOLOGY INCOLOGY PARTICLE OF THE LANCET COLOGY INCOLOGY INCOLOGY

 Presentation of results in national and international congresses.



9. Financial expression of the project

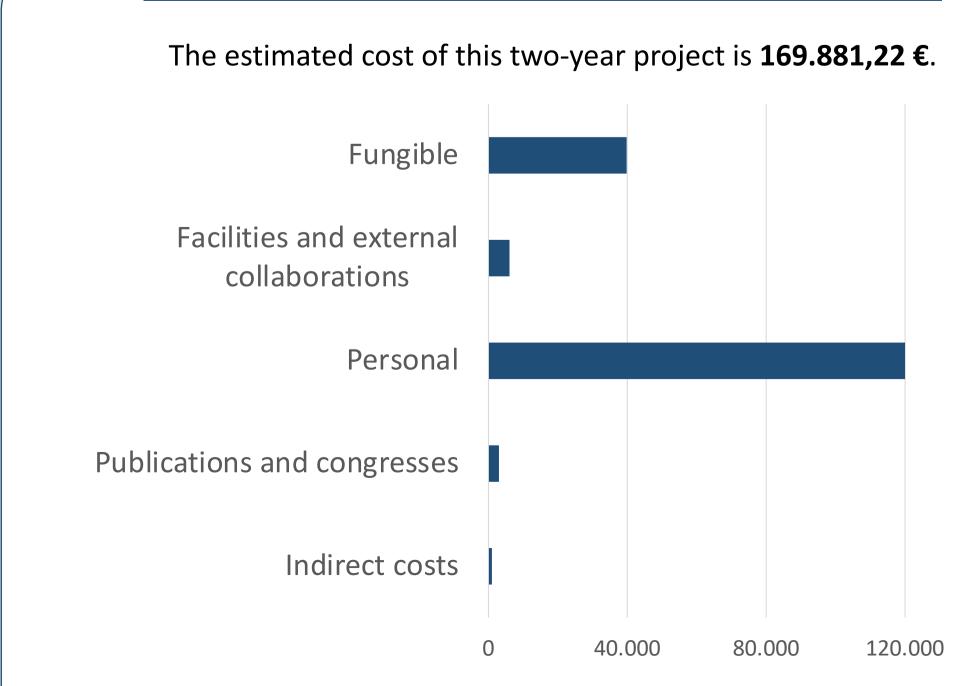


Figure 4. Graphic representation of the cost of the different items included in the budget.