The role of UPF1 cap-independent translation in colorectal cancer

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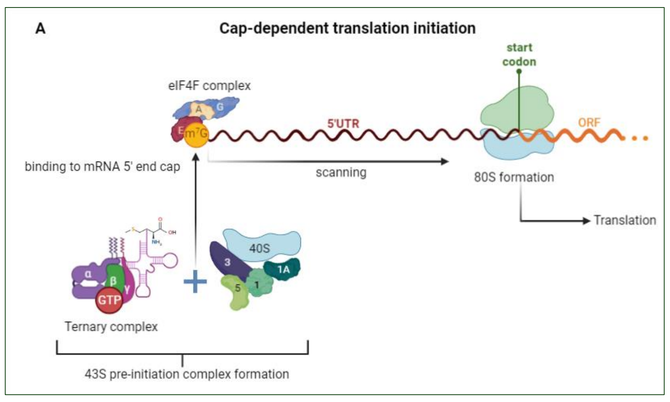
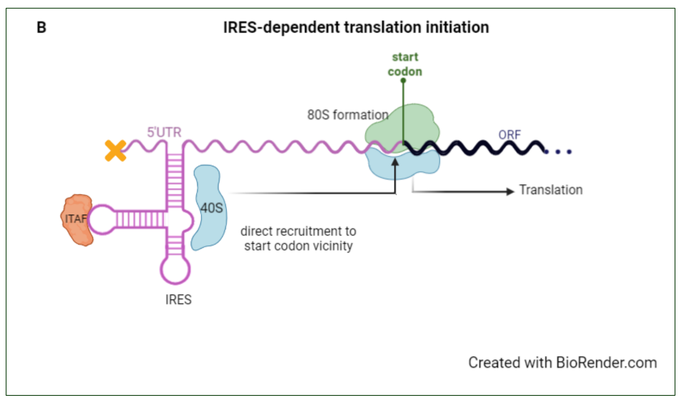
Background

Colorectal cancer

- Colorectal cancer (CRC) is the second most common cause of death worldwide
- 9.4% of all cancer-caused deaths
- Projections for 2040 show an increase in incidence and death rates





Role of UPF1 in the cell

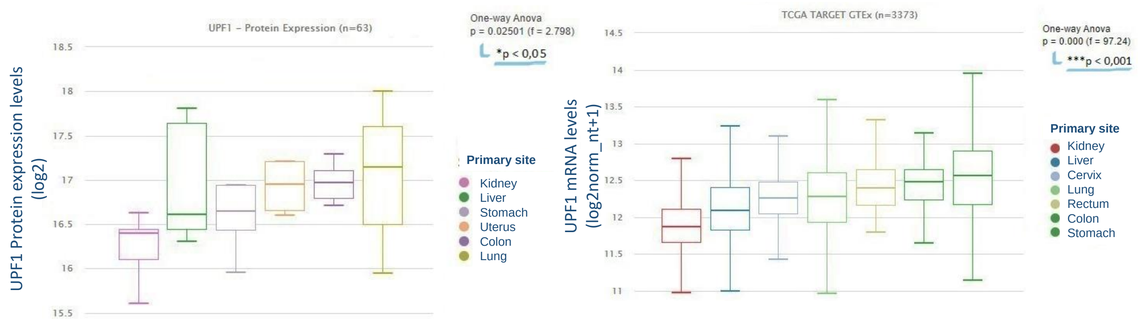
- RNA and DNA helicase
- Major player in nonsense-mediated mRNA decay (NMD)
- Crucial role in telomere replication and telomere length homeostasis
- Essential for cell cycle progression
- Tumour suppressor in several cancers
- Oncogene in colorectal cancer

Several proteins are able to maintain their expression via non-canonical mechanisms of translation initiation such as those synthesised by internal ribosome entry sites (IRESs) even under stress conditions

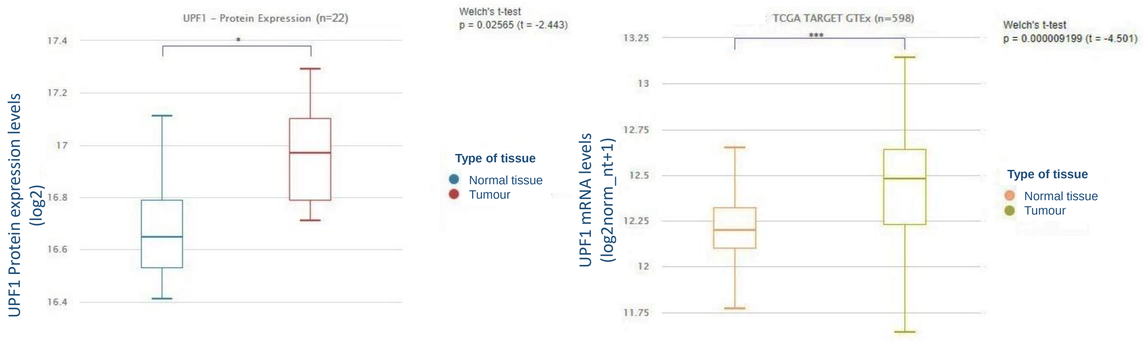
AIM

Investigate the biological importance of UPF1 IRES-mediated translation in colorectal cancer tumorigenesis

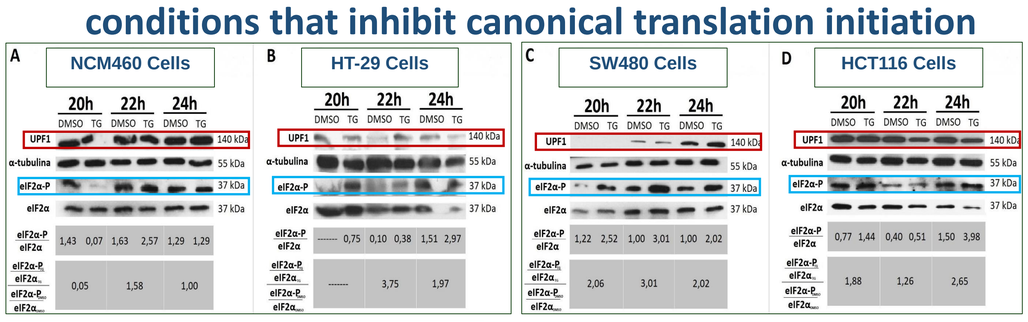
UPF1 protein and mRNA levels are greater in colorectal cancer than in other cancers



Colorectal cancer presents greater UPF1 protein and mRNAexpression levels than the counterpart normal tissue

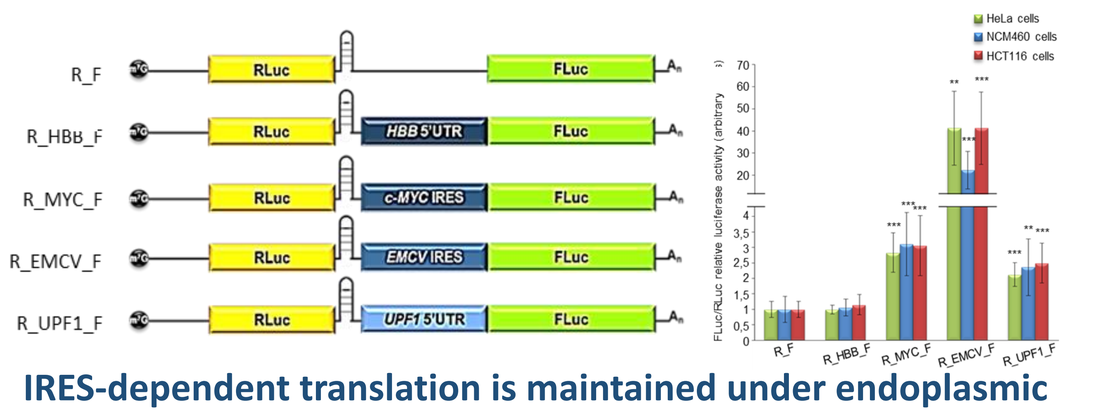


Expression of endogenous UPF1 is maintained under stress conditions that inhibit canonical translation initiation

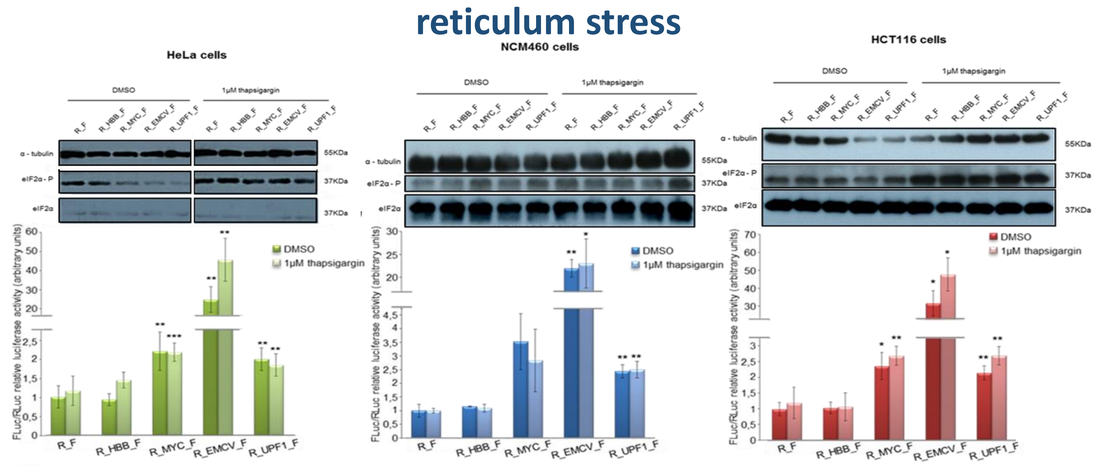


TG = thapsigargin, induces endoplasmic reticulum stress

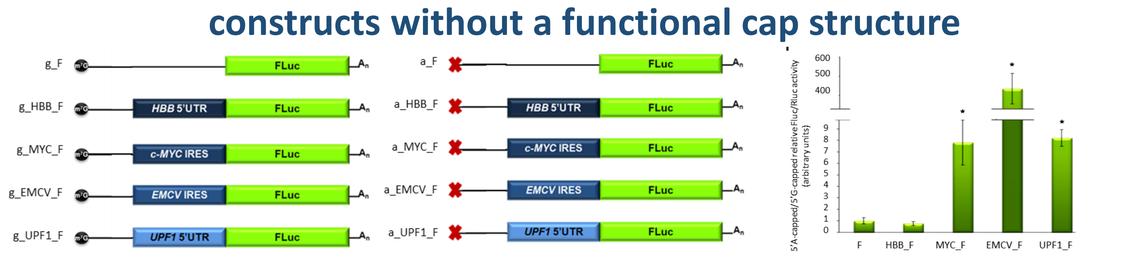
UPF1 5’ untranslated region (UTR) mediates IRES-dependent translation in a bicistronic construct



IRES-dependent translation is maintained under endoplasmic reticulum stress



IRES-dependent translation is active in monocistronic constructs without a functional cap structure



Material and Methods

We used HeLa (cervical cancer), NCM460 (normal intestinal mucosa), and HT-29, SW480 and HCT116 (different stages of colorectal cancer) cells were cultivated with the corresponding culture medium supplemented with 10 % foetal bovine serum, according to ATCC information. Cells were transfected with Lipofectamine 2000® and treated with 1 µM of thapsigargin or the corresponding vehicle (DMSO) for the indicated times. *In vitro*-transcribed mRNAs were produced from 1 µg of the corresponding plasmid previously linearised with *ClaI* restriction enzyme. Luminometry assays were performed in a 96-well plate luminometer with 10 µl of cleared lysate and 40 µl of each substrate. Western blot was performed according to standard procedures, using rabbit anti-UPF1, rabbit anti-phosphorylated eIF2α, rabbit anti-eIF2α and mouse anti-α-Tubulin antibodies overnight at 4 ºC with gentle shaking. After incubating with the corresponding secondary antibodies, membranes were developed with enhanced chemiluminescence reagents. We used 3 µM of each morpholino and 6 µM of Endo-Porter per well. *In silico* analyses were performed in the Xena platform, using data from The Cancer Genome Atlas (TCGA) and the Genotype-Tissue Expression (GTEx) databases for mRNA analyses and from the study of Zhou et al. (2020) for protein analyses. For further details on methods, please contact Rafaela Lacerda (rafaela.santos@insa.min-saude.pt)

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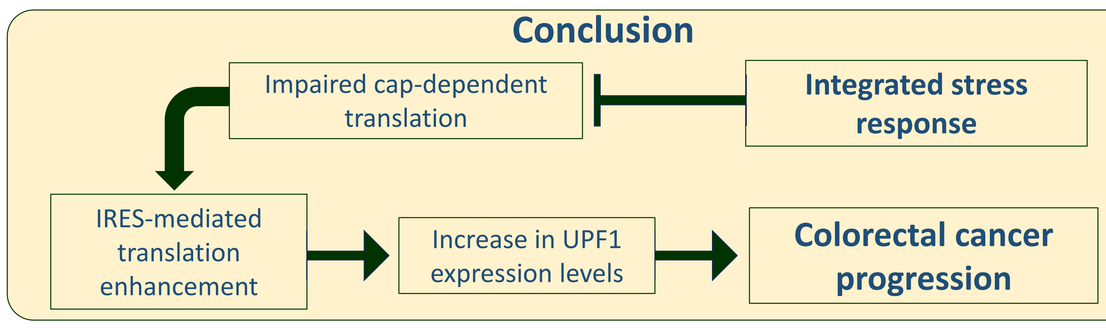
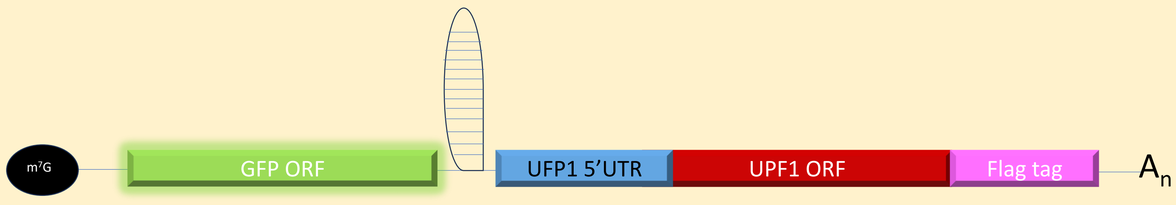
Conclusion



Ongoing work



Exogenously express UPF1 through its IRES from an *in vitro* transcribed bicistronic mRNA in cells under stress conditions inhibiting cap-dependent translation initiation and evaluate its impact in cell cycle progression and NMD efficiency