The role of methionine 1-linked ubiquitination in colorectal cancer progression in 3D cancer models

B.Sc. Virtanen Emmi[1], Ph.D. Martínez Chacón Gabriela [2], Doc. Meinander Annika[2]

[1]Department of Life Technology, University of Turku
[2]Faculty of Science and Engineering, Åbo Akademi University

MOLECULAR CELL BIOLOGY

Introduction

Colorectal cancer (CRC) is the third most common cancer in both men and women in Finland. Persistent and dysregulated inflammation is known to influence CRC as patients with chronic inflammatory bowel disease present the greatest predisposition. Overactivated NF-κB signalling seems to be one of the most important mechanism promoting both chronic inflammation and cancer progression.

Activation of NF-κB transcription factor family induces the expression of cancer promoting cytokines, such as TNFα and IL-6, and survival genes. During inflammation, NF-κB activation is tightly regulated by several post-translational modifications, especially by ubiquitination. Methionine 1-linked ubiquitin (M1-Ub) chains generated by LUBAC complex (HOIP, HOIL-1 and SHARPIN) are known to positively regulate NF-κB signalling. (Fig. 1).

However, it is yet to be known whether M1-Ub could have a role in cancer development. We hypothesize that increased levels of M1-Ub could drive CRC progression and inflammation. According to our hypothesis by targeting M1-Ub assembly complex (LUBAC), it could be possible to prevent and/or inhibit CRC progression in 3D cancer models.

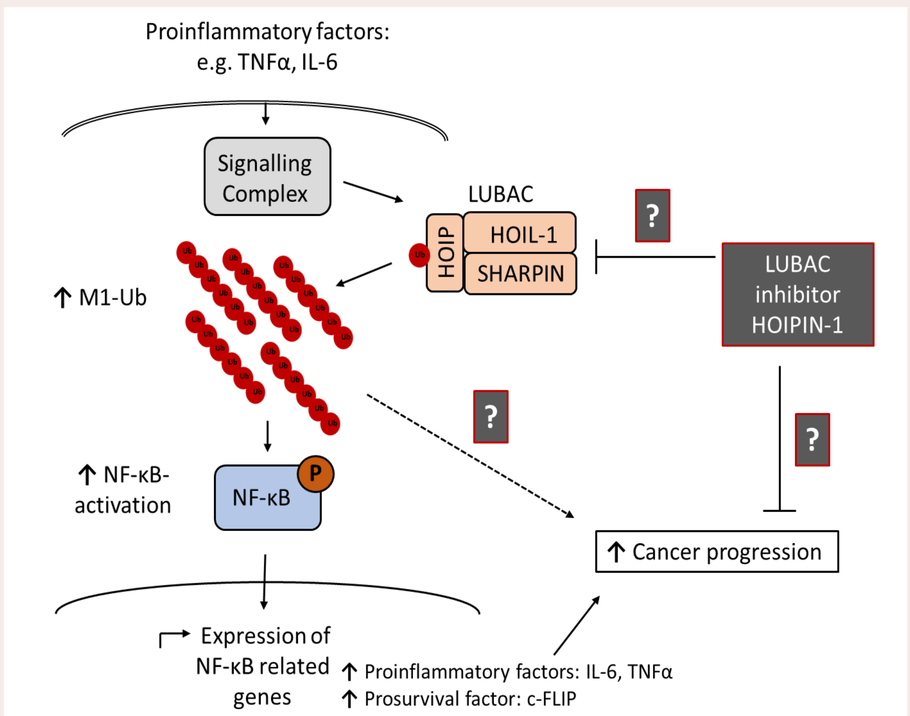
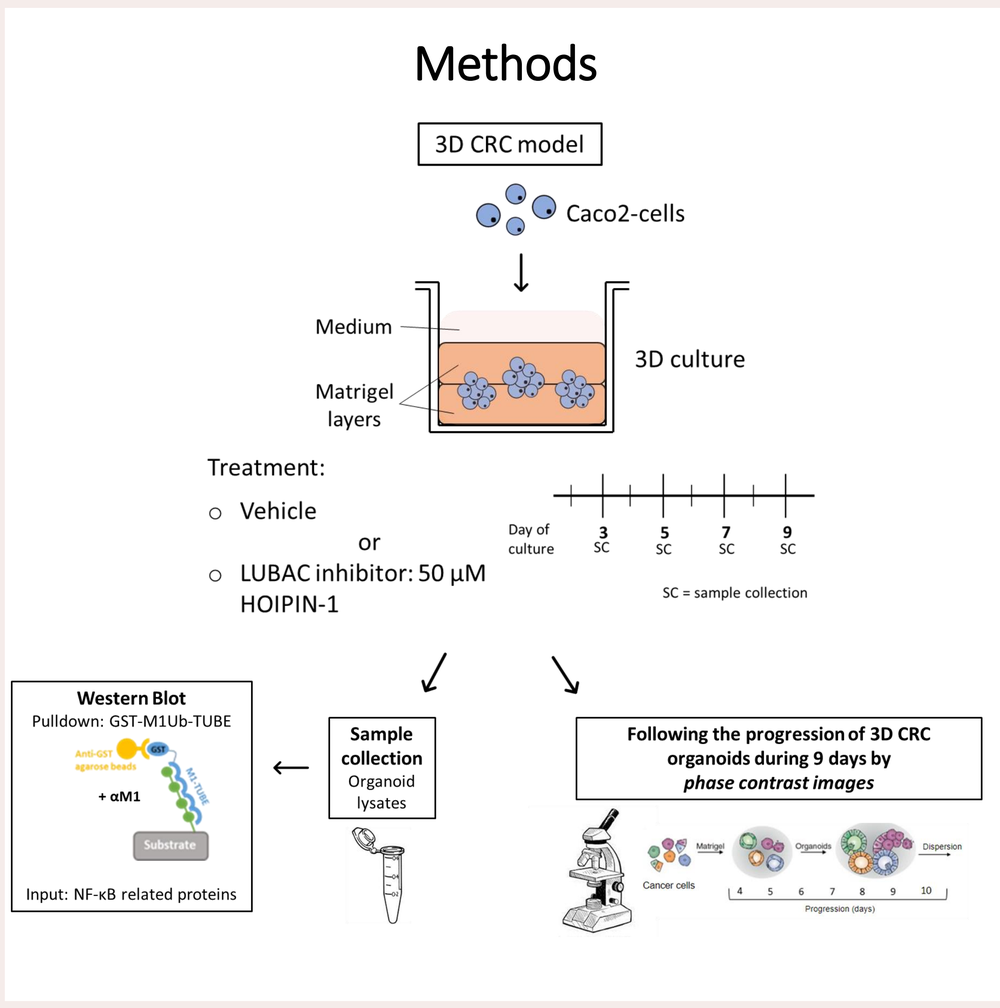


Figure 1. M1-Ub regulates NF-κB signalling activation and expression of NF-κB related genes.

Aims

- Optimize the culture of CRC 3D organoids to assess the different stages of progression.
- Determine M1-Ub levels during CRC progression in 3D cultures.
- Evaluate the efficiency of HOIPIN-1 (HOIP inhibitor) to prevent M1-Ub formation and disrupt CRC progression in 3D cultures.

Methods



Results

M1-Ub levels increase during CRC progression in 3D cancer models

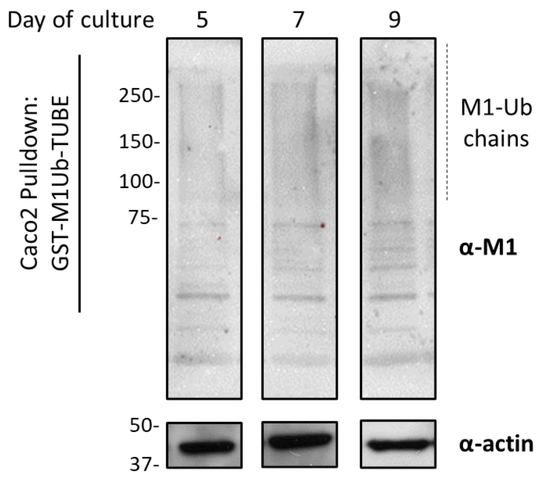
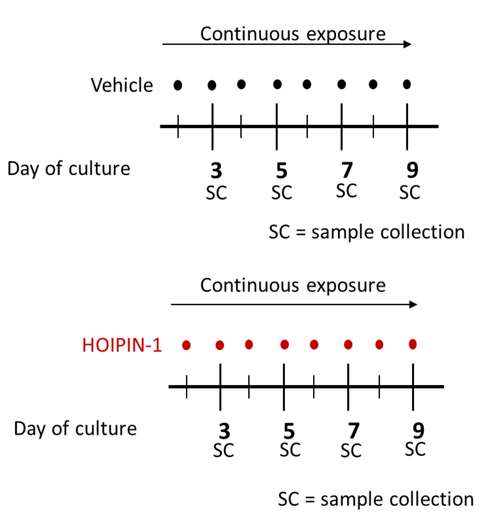


Figure 2. M1-Ub levels during CRC progression in 3D cancer models.
Western Blot with Caco2-cells. M1-Ub chains were isolated at denaturing conditions from organoid lysates with M1-TUBE. Ubiquitin chains from samples were analysed by Western blotting with α-M1 antibody, actin is used as a loading control.

Continuous inhibition of M1-Ub chain formation with HOIPIN-1 treatment leads to reduced levels of M1-Ub and NF-κB induced proteins



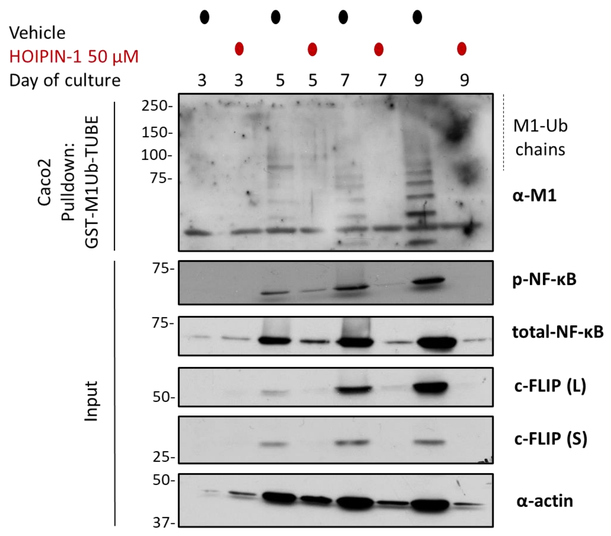


Figure 3. Experimental design. Samples were treated every two days with DMSO or 50 µM HOIPIN-1. Samples were collected on day 3, 5, 7 and 9.

Figure 4. Effects of HOIPIN-1 on M1-Ub and NF-κB-induced proteins during CRC 3D culture progression. M1-Ub chain were isolated similarly than in figure 2 and input samples were isolated at denaturing conditions from organoid lysate. Samples were analysed by Western Blot with specific antibodies and actin was used as a loading control.

Continuous treatment with HOIPIN-1 disturbs CRC progression leading to cell dead

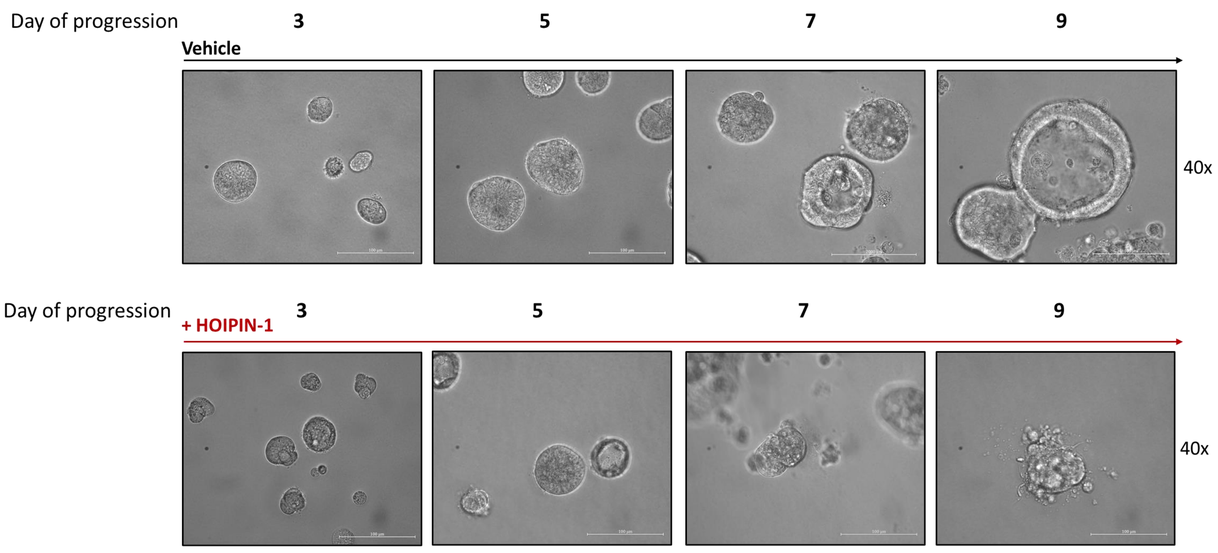


Figure 5. Phase contrast microscope images from progression of Caco2 tumor organoids. Samples were treated continuously either with DMSO or HOIPIN-1 and samples were collected on days 3, 5, 7 and 9. Continuous treatment with HOIPIN-1 disturbs CRC progression leading to cell death.

Conclusions

- M1-Ub levels are elevated in CRC organoids, particularly at advanced stages of progression.
- Continuous treatment with the LUBAC inhibitor, HOIPIN-1, inhibits M1-Ub chain formation during the progression of CRC organoids thereby altering NF-κB signalling activation.
- Inhibition of M1-Ub resulted in disturbances during CRC organoid formation, promoting cell death.
- **These results suggest that M1-Ub may have a crucial role in CRC tumor survival and cancer initiation and progression.**