Victorious

Patent enhancement of kojic acid derivative as selective inhibitor of mitosis in colorectal and glioblastoma cancer cells

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The primary method used to pharmacologically arrest cancer development and its metastasis is to disrupt the cell division process. Despite intensive studies on new chemotherapeutics, the biggest problem remains the side effects associated with the inhibition of cell division in non-tumoural host cells. The best anticancer strategy would include using a small molecule that targets and arrests cell division only in cancer cells. Here, we present the L1 molecule as a selective inhibitor of cell replication in cancer cells.

Introduction

Here, we introduce a novel small molecule L1 (**Figure 1**), which selectively inhibits mitosis solely in tumour cells. L1 is a derivative of kojic acid. In our previous studies [1], it was shown that L1 complexes with iron(III) ions bind to DNA, leading to inhibition of polymerase reaction. The mechanism of mitosis inhibition by L1 is distinct from that of first- and second-generation antimitotics, leading to apoptosis.

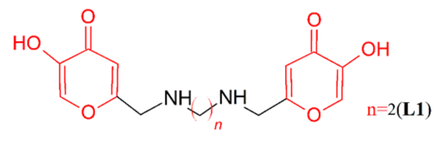

Figure 1. Molecular structure of L1 molecule. Kojic acid units are highlighted in red.

The morphological studies (**Figure 2** and **3**) conducted on different cell lines showed that the mitosis inhibition process, inducted by L1, is limited to tumoral cell lines. The molecular analysis with the use of Next Generation Sequencing confirmed the morphological analysis. The heatmap analysis of DE genes showed substantial differences in gene expression between non-treated Caco2 cells and cells treated with L1 (**Figure 4**).

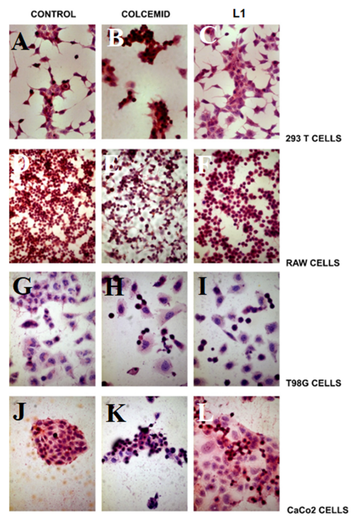

Figure 2. H&E staining of non-tumoural and two tumoural cell lines observed at ×10, ×20, ×40 magnifications (A-C) HEK293T, (D-F) RAW, (G-I) T98G and (J-L) Caco2 cell lines, respectively. Panels (A,B,G) and (J) depict non-treated cells (control samples). Panels (B, E, H, K) show the Colcemid-treated cells (positive control samples), while the panels (C,F,I,N) show cells treated with L1 (0.74 mM) for 24 hours.

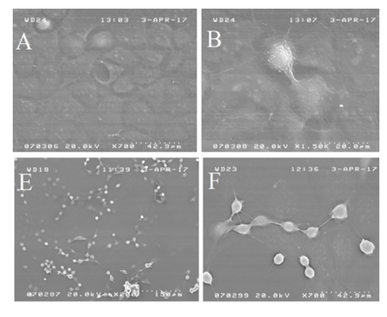

Figure 3. SEM images of (A-D) non-treated Caco2 cells. (E-H) Caco2 cells treated with L1 (0.74 mM final concentration in the cell growing medium; 24 hours).

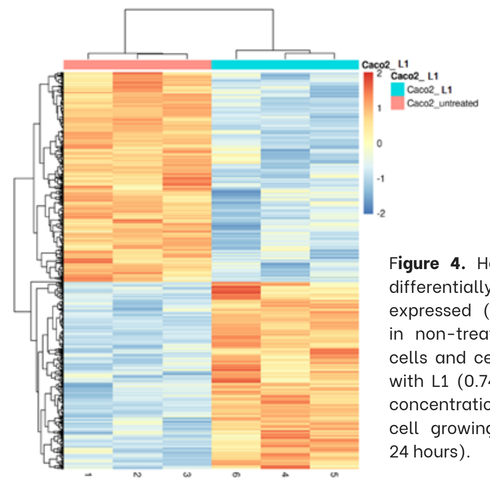

Figure 4. Heatmap of differentially expressed (DE) genes in non-treated Caco2 cells and cells treated with L1 (0.74 mM final concentration in the cell growing medium; 24 hours).

Objective

The main objective of this project is to strengthen the TRL 5 of our invention through further studies on tumor and non-tumor tissues collected from colorectal cancer (CRC) patients.

Increasing additional scientific data is necessary to initiate future ethical procedures and funding applications for xenograft model studies (TRL6) and pre-clinical studies (TRL 7).

Methodology

Normal and neoplastic human colon or rectal tissues were obtained at surgery. The specimens were processes accoridng to the Dame colon tissue organ culture [2].

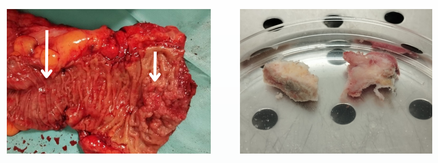

Figure 5. Resection of human tissue at surgery (left) and tumour tissue before organ culture. The mucosa-submucosa is isolated from the thick muscularis propria by first anchoring the mucosa with a haemostat, then gently teasing away the muscularis propria by slicing down the margin between the tissue fronts.

Results

In 12 months, the tissues of 22 CRC patients were processed for the L1 organ culture.
Zero-time (T0) control tissue and 24 h-incubated tissue from organ culture were further analysed by H&E stianing, SEM microscopy, NGS and CytoFlow analysis.

The obtained results showed distinct effects of L1 tratment on normal and neoplastic tissues.

Analysis

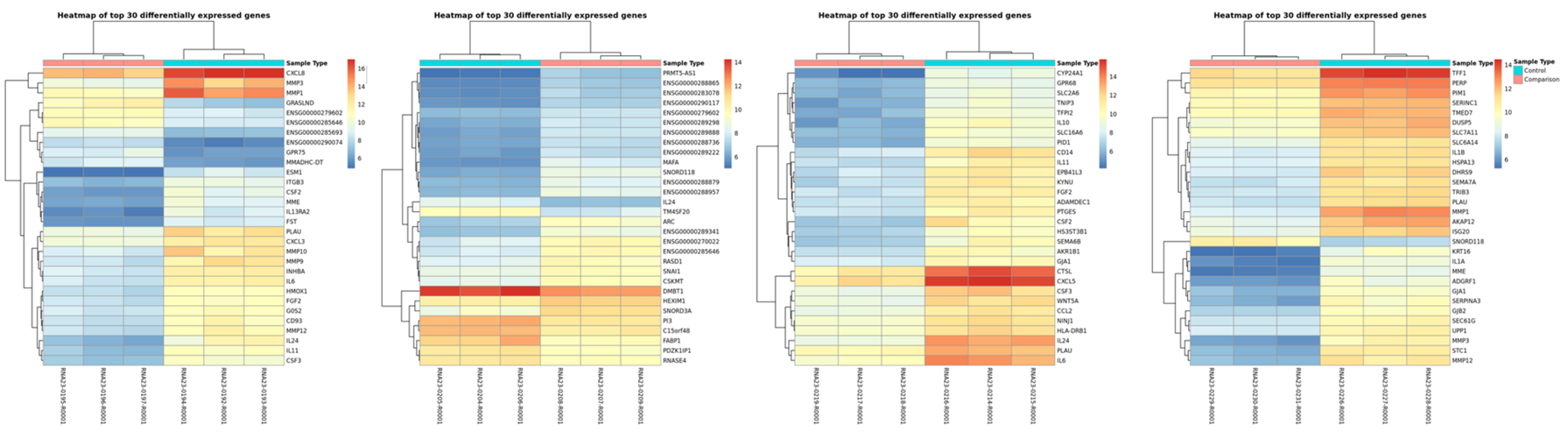

Figure 6. Heatmap of differentially expressed (DE) genes in non-treated and treated with L1 human colorectal tissues in 4 patients with CRC.

The NGS analysis has shown that the treatment has an effect on the modulation of gene expression (**Figure 6**). The prevailing trend is towards downregulation.

The NGS results were corroborated by the morphological analysis carried out by means of Scanning Electron Microscopy, SEM (**Figure 7**), which show a progressive reduction in cell overgrowth after treatment with L1.

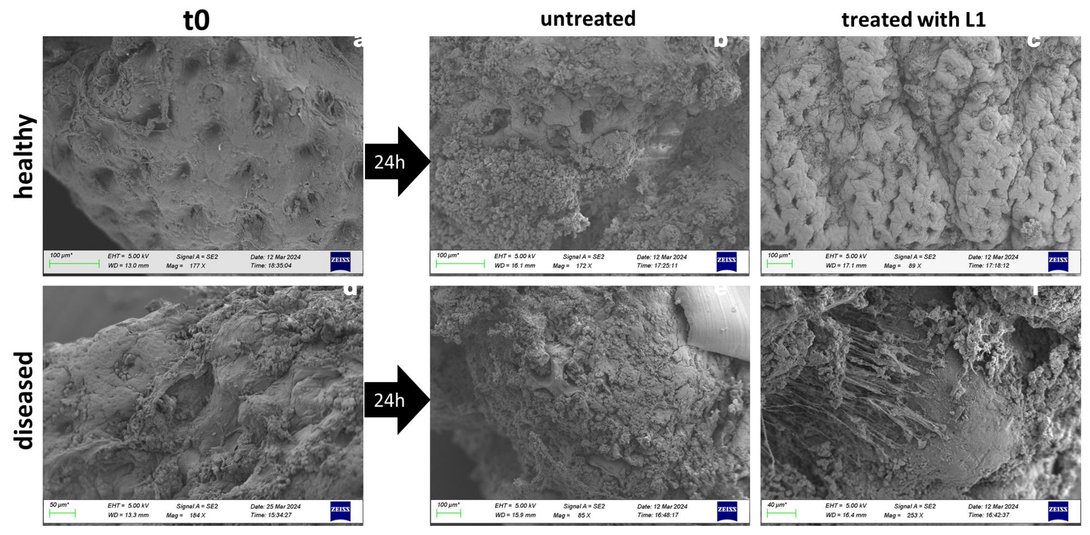

Figure 7. SEM images of healthy and diseased tissues ***a,d)*** freshly extracted, ***b,e)*** 24 hours after the biopsy in culture medium, and ***c,f)*** 24 hours after the biopsy in culture medium containing the L1 molecule.

Conclusion : the L1 molecule and the VICTORIOUS project experience

L1 is a novel molecule that specifically induces mitotic arrest in cancer cells. Extensive studies conducted on both non-tumoural and tumoural cell lines have consistently shown that mitotic arrest occurs exclusively in cancer cells treated with L1.
The mechanism of L1 action is selective, which differs L1 from other known antimitotic agents, positioning it as a critical third generation of antimitotic compound.

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VALMA-CO is the acronym of the project behind VICTORIOUS, currently funded by the University of Cagliari (PoC 2022).

Related Literature

[1] Lachowicz, Joanna I., et al. "Kojic acid derivatives as double face ligands for metal and phosphate ions." Journal of Inorganic Biochemistry 222 (2021): 111520.
[2] In Vitro Cell Dev Biol Anim. 2010 February; 46(2): 114-122. doi:10.1007/s11626-009-9247-9.

Patents

The presented here invetion was patented:
Kojic acid derivative as selective inhibitor of mitosis in colorectal and glioblastoma cancer cells; WO EP US CA IT CA3192894A1

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