“Effect of HDAC6 inhibition on the expression of genes involved in the immune response in colorectal cancer cells”

Rivas-Valdés Fernando[1 2], Mardones-Molina Constanza[1], Navarrete-Muñoz Camila[1,3], Hepp Matias[1]

[1]Laboratorio de Investigación en Ciencias Biomédicas, Facultad de Medicina, Universidad Católica de La Santísima Concepción, Concepción, CL [2]Tecnología Médica, Facultad de Ciencias de la Salud, Universidad San Sebastián, Concepción, Chile.

[3]Universidad de Concepción, Concepción, CL, **(frivas@ucsc.cl)**

ABSTRACT

Colorectal cancer (CRC) is one of the most common malignant neoplasm in Chile and according to the WHO, represents the second cause of death from tumors in the world. Current therapies include surgery, chemotherapy, radiation therapy and, recently, immunotherapy aimed at inhibiting the immune checkpoints, as Programmed Dead 1 (PD-1) receptor, that interact with his ligand (PD-L1) in the tumor. However, there is a percentage of patients who don't respond to this therapy, making it necessary to search for alternative or adjuvant treatments. Histone deacetylases (HDAC) have been positioned as candidates in the treatment of cancer, since they modulate the immune response, and being involve in tumor progression. Here, we investigated the effect of HDAC6 inhibitors on the expression of genes involved in the immune response. For that, Nexturastat A (NextA) an HDAC6i, was used to treat CRC cells. A time lapse with high concentration of NextA showed decrease in the expression of PD-L1. Also, to emulate an inflammatory environment, we treat the cells with Interleukin-6 (IL-6) and with or without NextA, observed a very marked increase in the expression of PD-L1 where induce with IL-6 and a clear decrease in the expression of PD-L1 with dual treatment in both CRC cells. Therefore, HDAC6 can by suggested as an interesting target as an adjuvant in CRC immunotherapy.

INTRODUCTION

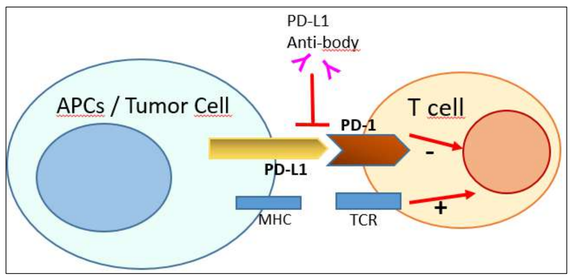


Fig 1. Interaction between PD-1 with its ligand (PD-L1) in T-cells and APC/tumor cells. PD-L1 is normally expressed in APCs to limit self-immunity. But, in cancer there is a high expression of PD-L1 in tumor cells to evade the immune system and suppress the activation of T cells. Immunotherapy with PD-L1 blocking antibodies enhances the immune response against tumor cells, but generates resistance. That generate more expression of new molecules of PD-L1 on the cell surface.

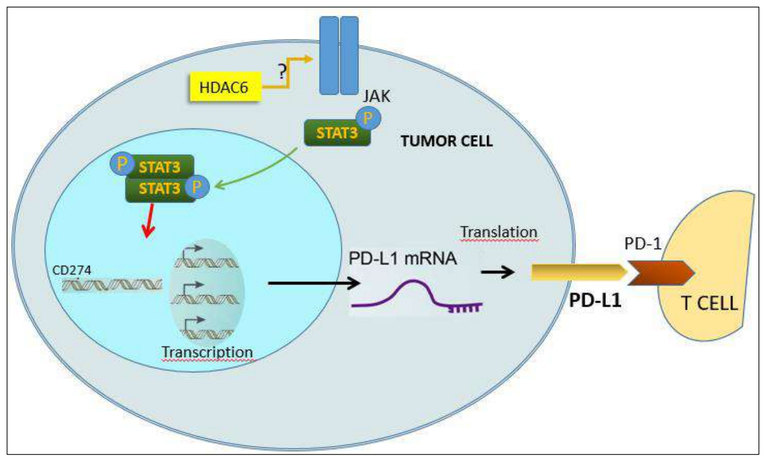


Fig 2. Representative scheme of PD-L1 transcriptional regulation by STAT3 transcription factor. HDAC6 is involved in STAT3 phosphorylation, then phosphorylated STAT3 homodimerizes and translocates to the nucleus to activate transcription of its targets. Cytoplasmic HDAC6 can deacetylate or modulate members of the STAT3 activation pathways.

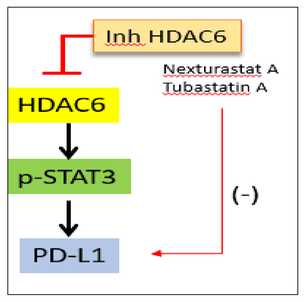
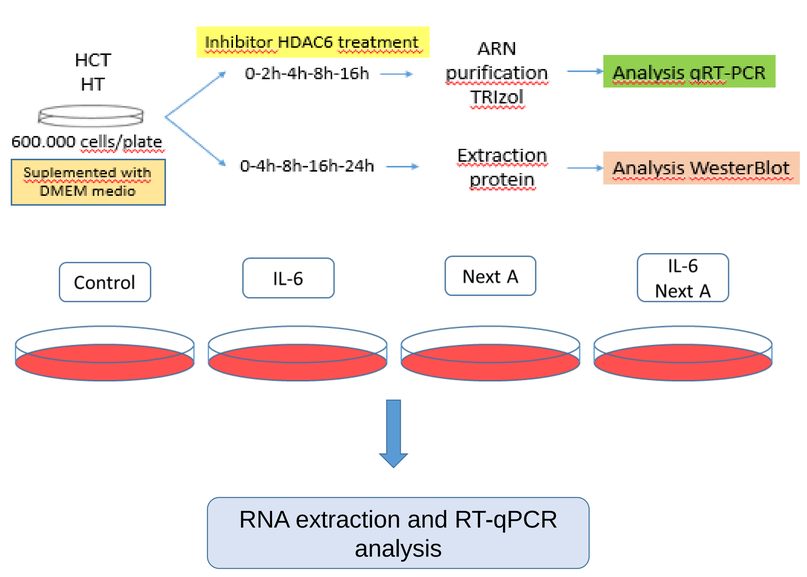
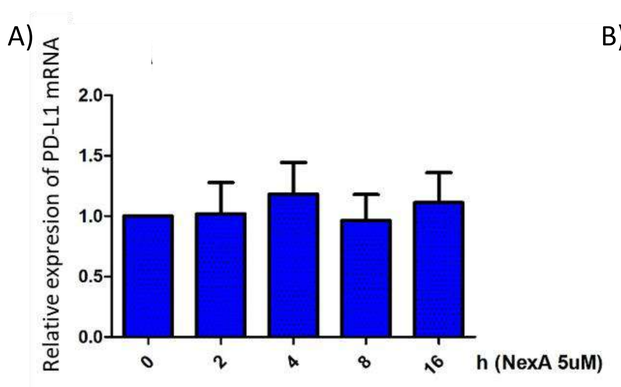


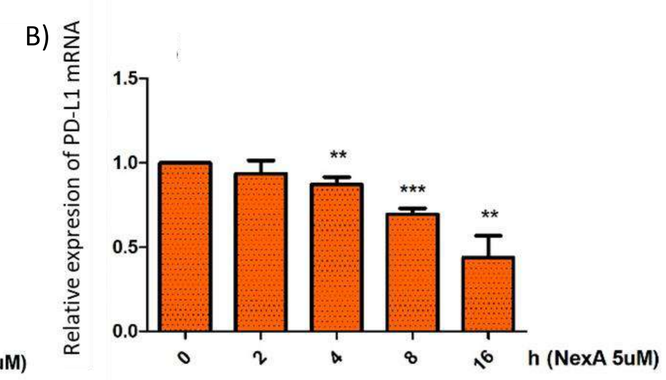
Fig 3. HDAC6 controls the activation of STAT3 and the expression of PD-L1. It has been reported that HDAC6 inhibitors indirectly modulate the expression of PD-L1 in other types of cancer through STAT3 activation, decreasing PD-L1 expression. This is unknown in colorectal cancer.

METHODS



RESULTS





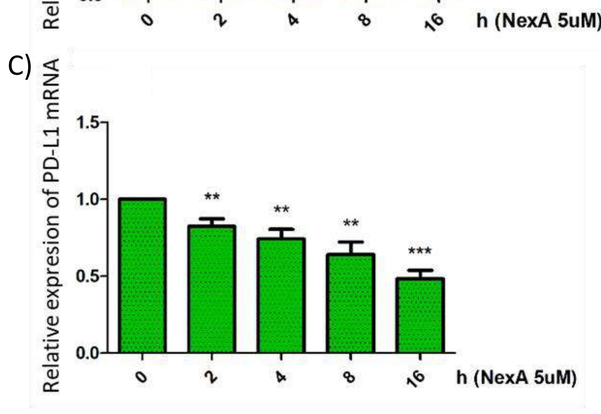


Fig 4. Relative expression of PD-L1 levels. Relative quantification of PD-L1 mRNA after treatment with 5uM Nexturastat A at different exposure times in CCD841 (A), HT29 (B) and HCT116 (C) cell lines. Results are the average of 3 independent experiments. Statistical analysis by t-Student (significance values * = p <0.05; ** = p <0.01; *** = p <0.001). The relative quantification was calculated using 2ΔΔCT with 18S as the reference gene.

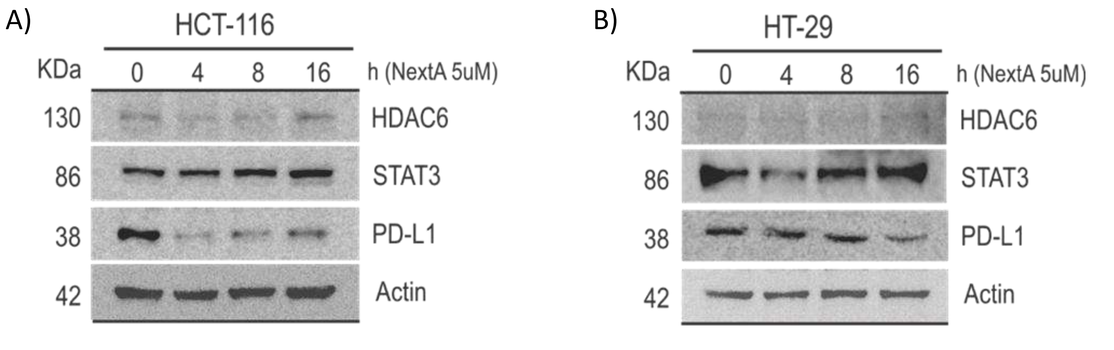
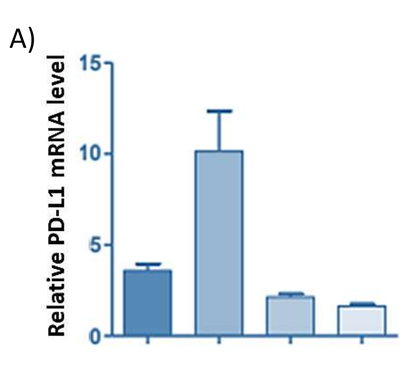
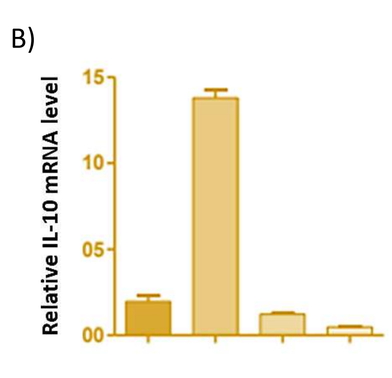
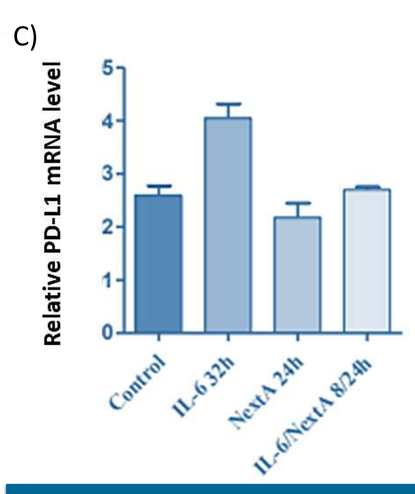


Fig 5: Expression of different protein involve in PD-L1 regulation. Protein expression for colorectal cancer cell lines at different times of treatment with 5uM of HDAC6 inhibitor**.** Colorectal cancer cells (A) HCT116 (B) HT29. Analyze by Western Blot.







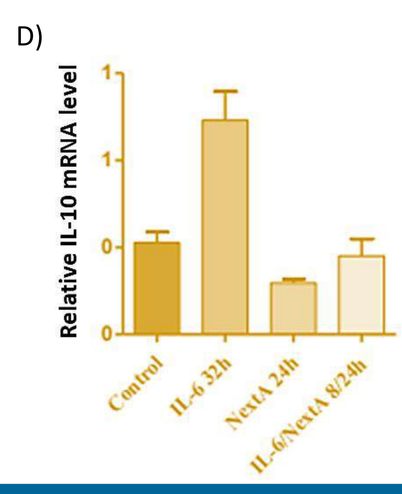


Fig 6. Relative quantification of PD-L1 mRNA expression levels (A and C) and IL-10 (B and D) in colorectal cancer cells. Combinatorial treatments were performed with IL-6 and Nexturastat A, to verify PD-L1 and IL-10 expression in HT-29 cells (A and B) and HCT-116 (C and D). The drugs were used in the following way; IL-6 by 32 hours, NextA for 24 hours and NextA was added to combination at 8 hours post IL-6, leaving it for 32 total hours. Results are the average of 3 independent experiments. The relative quantification was calculated using 2ΔΔCT with 18S as the reference gene.

CONCLUSIONS & FUTURE PROJECTIONS

- Nexturast A, specific HDAC6 inhibitor, can modulates the expression of PD-L1 indirectly. Particularly produces a decrease in the PD-L1 expression at mRNA and protein levels.

❑ Nexturastat A can by suggested as an adjuvant drug in CRC immunotherapy, but more research is needed.